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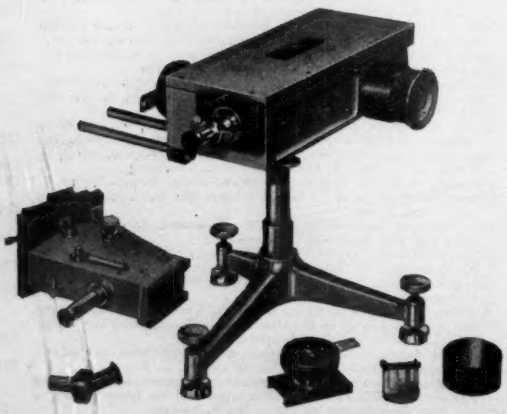

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SCIENTIFIC AND INDUSTRIAL RESEARCH: THE STATE AND INDUSTRY

IN an earlier article (*Nature*, October 25, p. 1111), a general indication was given of the activities of the Department of Scientific and Industrial Research, and the relations of the Department with other government departments interested in aspects of scientific and industrial research were discussed. We now come to consider the relations of the Department's work with the research associations and industry, in the light of the comments made in the fifth report for the session 1957–58 of the Select Committee on Estimates*.

The Estimates Committee confines its criticism to two aspects: information services and financial recoveries from industry. While in general it is satisfied that the proportion of effort devoted to information services, at present about a quarter of the work of all the establishments, is a reasonable one and likely to achieve good results, it found two weaknesses. Stressing the importance of an individual approach under modern conditions, it believes it is a mistake not to approach individual firms which might be expected to be interested in particular results, and recommends that officers of establishments should be encouraged to write to, and to visit personally, individual firms or associations representing a particular industry likely to be interested in any results achieved by the establishments. Secondly, it regards some of the publications of the establishments as too general or nebulous in character. Unless a publication is of obvious direct and specific interest, it has little chance of attracting attention, and it may even tend to discourage the reading of the Department's publications generally. The sound suggestion is made that, before embarking on any publication of its own, the Department should be perfectly clear as to who it wishes to interest by the information to be given, and design the publication accordingly.

Beyond this estimate that the Department as a whole is spending something of the order of £1–£1·25 million on information services, the report gives no indication of the actual expenditure on the Headquarters Information Division, and some of the activities of the latter which have been freely criticized by information officers and librarians escape notice. Nor is there any recognition in this report of the importance of the way in which such information services are geared to the national system of library and information services. Their efficiency depends very largely, in fact, on the way in which Government policy and practice maintain an adequate national system. A National Science Centre, a National Lending Library for Science and Technology and a National Reference Library in that field, as well as an adequate regional system, are vital factors, and appear to have been completely overlooked by the Select Committee.

As regards the financial recoveries by the establishments for work done for industry, the Select Committee on Estimates recognizes that these necessarily vary widely with different conditions, from 95·4 per cent for the Fire Research Station, which is a joint enterprise between the Department and the Fire Offices Committee, which bears half the cost of the Station, to as little as 0·3 per cent for the Radio Research Laboratory, which works mainly for Government departments. Any increased rate of recovery for this Laboratory, the Pest Infestation Laboratory, the Road Research Laboratory and the Water Pollution Research Laboratory accordingly is likely to be at the expense of another Government department; nor does the new work of the Fuel Research Station promise to offer much opportunity for recovering any substantial part of the cost, in any event for the present. On the other hand, the Committee considers that the recovery rates of the Food Investigation Organization (3·4 per cent), the Mechanical Engineering Research Laboratory (5·9 per cent) and the Building Research Station (6·5 per cent) are much too low. It regards the new scales of charges to industry which the Department has recently prescribed for the three stations as satisfactory and likely to lead to some improvement in the rate of recovery; but it recommends that all three stations should approach the question with greater determination to achieve the best possible rate consistent with departmental policy, and in particular that the Building Research Station should take account of the benefits which a supplying industry may receive when research connected with its products is successfully undertaken.

One further recommendation in this context is prompted by the measurements which the Building Research Station is making of the effect on the Bakerloo tunnel of removing and replacing the earth cover while the new Shell building in London is being constructed. This work is of great value to the Department in connexion with its study on tunnels and also to the Shell Co., in that it is helping to reduce the risk of damage to the tunnel. The Estimates Committee recommends that in future, where it seems possible that any work carried out by the Department for its own purposes may incidentally result in direct advantage to an individual firm, an agreement should be negotiated with the firm concerned before the work is started, whereby the firm should pay a direct contribution to the costs, if in fact the firm obtains a direct advantage as a result of the work.

* Fifth Report from the Select Committee on Estimates together with part of the Minutes of the Evidence taken before Sub-Committee B and Appendices, Session 1957–58—The Department of Scientific and Industrial Research. Pp. xvi+165. (London: H.M. Stationery Office, 1958.) 9s. net.

These recommendations of the Estimates Committee, and those already mentioned (*Nature*, October 25) in dealing with the relation of the Department of Scientific and Industrial Research with other Government Departments, have far-reaching implications which are not discussed in the report, and it seems doubtful whether the Committee fully appreciates the import of some of its proposals. It is at least arguable whether the Committee's approach to the whole question of receipts for work done by the Department is sound. It seems to ignore the question of national advantage, and to overlook the fact that research is a resource and not simply an investment. The returns, moreover, are not to be reckoned in financial terms, and while, on the evidence submitted to the Committee, it is probable that some of the establishments of the Department might make firmer efforts to secure some greater return when work has been carried out for the benefit of a particular institution, firm or department, it is at least dubious whether that should be a prime consideration in research policy.

In the first place, the instructions recommended by the Committee are likely to lead to unnecessary delay in carrying out a research programme which the Department wishes to implement for its own purposes; agreements are unlikely to be negotiated readily or quickly, particularly if the benefits are intangible or a long way ahead. Secondly, the outlook which the Estimates Committee seeks to foster could easily distort the balance of research carried out by the Department by encouraging concentration on *ad hoc* or sponsored investigations and inquiries rather than on the long-range investigations which should be a prime purpose in a central research organization. Apart from this, it overlooks the educational value of the Department's research stations in encouraging research in industry. Excessive concentration on the financial side could easily hinder the attempt which the Department is making to foster a spirit of research and co-operation with industry, and it also ignores the fact that the benefits which accrue from the Department's successful investigations are in fact returns to the nation as a whole and part of the justification of its investment.

The very fact that the work of the Department has been submitted to this searching inquiry by the Estimates Committee points to the need for some satisfactory means of setting forth, both to Parliament and to the general public, a clear and balanced picture of the work of the Department as a whole, and its significance both for the national economy and public welfare. For all the shrewd comments in this report, it appears to have escaped the Select Committee that this problem is still unsolved. Again, while the minutes of evidence contain much that deserves to be noted and weighed by the scientist and by those concerned with the organization of the nation's scientific and technical effort, the Estimates Committee, even when due regard is had to the limits of its terms of reference, seems to have been guided by too narrow a conception of efficiency as applied to scientific and technological effort.

The efficient use of the limited resources in Britain of scientific and technological man-power, which is the decisive factor in that effort, depends on the wise selection of objectives and, as the Estimates Committee recognizes, on the appropriate termination of projects and programmes, though this may well be less a financial or economic decision than it appears to indicate. It depends no less, however, on the ability to attract men of the highest calibre; it also depends on the conditions under which they are required to serve, so that there is neither waste of highly trained man-power on work which could be equally well performed by less highly trained men nor uneconomic use of highly expensive equipment. Some of the Committee's recommendations seem likely to discourage recruitment of staff of the requisite calibre, and to lead to expensive equipment being insufficiently used. Certainly they are unlikely to encourage the maximum use of such equipment.

Nevertheless, the comments should prompt a further and widespread discussion of the whole structure and balance of the nation's research effort, and its disposition between Government establishments, universities and industry. It provides an opportunity which should be used by professional bodies as well as by Parliament, not merely to ensure that the administrative structure is appropriate and effective, but also that the ancillary factors contributing to the effective use of the scientific and technological effort of Britain, as well as to the efficiency with which that effort is conducted, receive adequate attention and support. It is probably at that point above all that additional financial support could bring the biggest increase in the return from the national effort in research and development at the present time. The Estimates Committee's report may well direct further attention to such matters of organization as the departmental organization of research in relation to a general or central body such as the Department of Scientific and Industrial Research; but it is this question of the contributory factors on which attention needs to be focused.

FLUID MOTION IN NATURE

Natural Aerodynamics

By Dr. R. S. Scorer. (International Series of Monographs on Aeronautical Sciences and Space Flight. Division 2: Aerodynamics, Vol. 1.) Pp. xi+312+22 plates. (London and New York: Pergamon Press, 1958.) 60*s*. net.

THE unusual title of this book is easily explained. Dr. Scorer writes about those motions of real fluids which arise chiefly as a result of solar heating and topography. The book is not concerned with the central problem of ordinary aerodynamics, the calculation of the forces experienced by bodies of prescribed shape moving through the air, but is an account of winds, convection currents, air waves, clouds and rain, and thus comprises a selection of topics from both dynamical and physical meteorology.

In the opening chapters, which deal with the equations of hydrodynamics and the concepts of

vorticity and viscosity, the treatment might fairly be described as 'popular', in that much of the account is in plain language and no excessive demands are made upon the reader's knowledge of mathematical techniques. The later chapters, which deal with specific problems, require a firm grip of applied mathematics for their understanding; but the book is addressed more to the physicist and meteorologist than the mathematical specialist, and the author evidently has decided that for his purpose physical ideas are more important than details of mathematical manipulation. The result is an unorthodox but lively text, for Dr. Scorer never hesitates to interpose philosophical arguments whenever an opportunity presents itself.

The first six chapters summarize the equations of fluid motion, the Coriolis effect, vorticity, viscosity, boundary layers, wakes and turbulence. The discussion of such matters in about 140 pages implies considerable condensation, and at least one theorem concerning circulation is stated incorrectly in its abbreviated form (p. 59). However, the average physicist and perhaps even the specialist in fluid motion can scarcely fail to gain some new insight into the ways of fluids from a perusal of these vigorously written chapters (especially that dealing with vorticity), although there are dangers of oversimplification, as in the facile definition of turbulence as "a kind of stirring motion by which anything possessed by air is diffused" (p. 124). This section of the book is a useful introduction to a subject which tends rather easily to become a preserve of mathematicians, and it is refreshing to be reminded that at times even the hypothetical ideal fluid of the classical hydrodynamics has affinities with air and water.

The real originality of the book and, in the reviewer's opinion, its main value, lies in Chapters 7–10, which deal with matters which, for the most part, have not yet found their way into the curricula of the universities, although their importance in many fields of applied science is manifest. Natural or buoyant convection usually gets little more than a brief reference in text-books, and then on a qualitative rather than a quantitative basis. Dr. Scorer's account of convection is primarily mathematical, but the practical application is well illustrated by examples from meteorology (cumulus clouds), gliding, and the behaviour of hot plumes from industrial stacks. The chapter on air waves ranges from explosions to lee waves set up by mountains, a topic on which Dr. Scorer is especially well qualified to write. The account of the formation of clouds and of precipitation is mainly descriptive, which is to be expected in view of the varied and highly complicated physical processes involved. In the final chapter, entitled "Philosophy of Method", Dr. Scorer manages to combine an account of the methods of dimensions and of small perturbations with a frontal attack on geographers.

The book is thus an original account of some parts of mathematical physics mingled with the author's highly individualistic views on science in general. For such a wide field, the serious student may consider the treatment too superficial; the general reader, on the other hand, may be hard pressed at times to find his way through a juxtaposition of description and calculation. But Dr. Scorer has succeeded in writing a book which is often illuminating and never dull, and in its pages he has brought together much material which hitherto has existed only in scientific journals. There is nothing quite like this book in meteorological literature, and it is to be hoped that its high price will not deter those who wish to learn about the modern approach to some of the most fascinating and important problems of meteorology.

O. G. Sutton

MULTIVARIATE ANALYSIS

An Introduction to Multivariate Statistical Analysis

By Prof. T. W. Anderson. (Wiley Publications in Mathematical Statistics.) Pp. xii+374. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1958.) 100*s*. net.

Some Aspects of Multivariate Analysis

By Prof. S. N. Roy. (Indian Statistical Series, No. 1.) Pp. viii+214. (New York: John Wiley and Sons, Inc.; Calcutta: Indian Statistical Institute; London: Chapman and Hall, Ltd., 1957.) 64*s*. net.

The Analysis of Multiple Time-Series

By M. H. Quenouille. (Griffin's Statistical Monographs and Courses, No. 1.) Pp. 105. (London: Charles Griffin and Co., Ltd., 1957.) 24*s*.

THESE three books do indeed possess the common property of containing subject-matter falling under the general heading of 'multivariate analysis', but they differ greatly, both in content and intent, among themselves. They are arranged, above, in order of a marked increase in specialization. Prof. Anderson's book is intended for the advanced college student, and has—among other desirable text-book features—a clear and thorough manner of exposition, examples in the text, and an ample supply of problems (but no model solutions). Prof. Roy's book describes a number of special topics in multivariate analysis to which he has applied some original ideas on the construction of tests and confidence intervals. This book is really a rather loosely connected series of monographs, technically of a rather advanced nature, requiring a good deal of previous reading for full appreciation, but yielding rewards in stimulation and interest. In the last book of the trio, Quenouille gives detailed consideration, accompanied by many numerical illustrations, to the application of more or less standard statistical techniques in one specific field—that of time series in which, at each epoch, observations are made on more than one variable.

Were it not for considerations of price, it would be expected that Prof. Anderson's would prove to be the most widely used of the three books. It provides, for the first time, a work suitable for the student wishing to obtain a sound appreciation of the whole field of multivariate analysis. Careful study of this book should leave few gaps to be filled—apart from those created by the present flow of papers on this subject. The first four chapters contain fundamental results in statistical theory, with special bias towards multiple regression, and give the reader an opportunity to become accustomed to the habitual use of matrices and the consequent slightly unfamiliar appearance of the text. The next chapters are on the generalized (Hotelling's) T^2 statistic, on the important 'classification problem' (also known as 'discriminatory analysis'), and on the (Wishart) joint distribution of variances and covariances. These are followed by an important chapter on multivariate extensions of the general linear hypothesis, or 'generalized analysis

of variance', and two chapters on special applications of these results. Chapter 11 is on analysis into principal components, on the lines developed by Hotelling. Chapters 12 and 13 introduce the ideas of canonical correlations and variables, and the associated problem of finding the joint distribution of the characteristic roots of a certain matrix. There is a brief concluding chapter outlining various special topics in multivariate analysis.

Prof. Roy's book effectively takes as its starting point the latter part—Chapters 12 and 13 especially—of Prof. Anderson's book. Roy's method of test construction leads to criteria based on the greatest and the least of the characteristic roots of a certain matrix, rather than the symmetric functions of these roots which are usually encountered in the more conventional methods of approach. Roy is able to show that the tests he proposes should be reasonably sensitive, but he does not compare, directly, the powers of competing tests in any particular case. Indeed, numerical illustration is notably lacking, and even significance limits for the new criteria are not given, although some have been computed. In this book there will also be found (*a*) the construction of sets of 'simultaneous confidence intervals' for a number of parameters, with joint confidence coefficient defined as the probability that every one of the confidence intervals contains the true value of its appropriate parameter, and (*b*) analysis of multiple contingency tables (described as 'non-parametric generalizations of analysis of variance and multivariate analysis'). The omission of numerical illustration avoids directing attention, in (*a*), to the fact that the strong condition required of the confidence coefficient must often be paid for by uncomfortably wide confidence intervals. About one-third of the book is occupied by nine appendixes containing mathematical results used in the main text.

Quenouille summarizes the problems considered in his monograph as follows (slightly abbreviating the original): (1) Definition of the structure of several interdependent time series, and deduction therefrom of their properties. (2) Inference of structure from population, or from observed, correlation properties. (3) Likely errors in specification of structure, and the effects of these errors on analysis. (4) Methods of estimating values of parameters, given a particular structure, and of testing whether a particular structure is appropriate. (5) Practical application of techniques derived from study of the above problems to observed series.

The programme so specified is carried out in a competent manner. There does not seem to be any attempt to make the treatment or discussion exhaustive—for example, the work of Rudra on the problem of discriminating between rival structures in the case of univariate series is not mentioned—but the author has produced an adequate text for anyone wishing to extend a moderate knowledge of univariate time series analysis into the multivariate field. The text is written with evident regard for students' difficulties, and numerical illustration is profusely provided. The omission of any reference to periodic series and periodogram analysis is unfortunate, since it is a rather sweeping assumption to suppose that all observed series may be usefully assigned a moving average or autoregressive structure, with which the monograph is largely preoccupied. A list of symbols, with meanings, is provided. Exception can be taken to the author's statement that E is the usual symbol for the operator with the property:

$$Ex_t = x_{t-1}$$

—this operator would usually be written E^{-1}.

Both Roy's and Quenouille's works suffer from a number of small misprints, mostly in suffixes, exponents, and similar positions. They are not likely to prove misleading to the informed reader, though the occurrence of a subscript $t - 1$ instead of $t + 1$ in Quenouille's equation (5.6.1) could cause some puzzlement.

The essential qualities of the three books are, briefly: Anderson: a sound text-book of traditional style in a new field; well produced, but expensive for its size. Roy: a book for the specialist—and only for a very special kind of specialist; consolidates material at present scattered in the literature, and may stimulate some further research. Quenouille: a readable book, repaying the effort of understanding the rather unusual notation; coverage of subject rather uneven, but not unduly so for a fresh subject.

N. L. Johnson

EIGENFUNCTIONS

Eigenfunction Expansions Associated with Second-Order Differential Equations, Part 2

By Prof. E. C. Titchmarsh. Pp. xi+404. (Oxford: Clarendon Press; London: Oxford University Press, 1958.) 70*s*. net.

In 1946, Prof. Titchmarsh published "Eigenfunction Expansions associated with Second-Order Differential Equations" (Oxford, at the Clarendon Press) and a photographic reprint was made in 1950. This slim and modest volume contained the results of Titchmarsh's researches from 1939 until 1945 and provided a new and original theory of this important and growing subject, which was applicable not merely to equations of the Sturm–Liouville type, but also to the singular equations studied by Weyl.

Titchmarsh's method is independent of the calculus of variations, of the theory of integral equations and of the theory of linear operators—each of which has been used as a basis of the theory of eigenfunctions of differential equations. Titchmarsh had discovered a simple and direct method of studying this subject by means of contour integration and the calculus of residues, and of proving the central expansion theorem. In the present volume this method is extended to *partial* differential equations of the form:

$$\Delta\psi + \{\lambda - q(x,y,\ldots)\}\,\psi = 0$$

where Δ is the Laplacian operator in n-dimensional Euclidean space.

The principal problems discussed are (1) the expansion of an arbitrary function in terms of the eigenfunctions of a prescribed equation, (2) the effect of small changes in the equation on the eigenvalues and eigenfunctions, (3) the nature of the spectrum and the distribution of the eigenvalues. In addition to these great questions, the author also considers a number of special problems of considerable interest, namely, the eigenvalues of separable equations, and the case in which the function $q(x,y,\ldots)$ is periodic. The final chapter deals with the analytical apparatus which has been used in the body of the work and provides proofs for the various theorems which have been required.

Many of these problems are of considerable interest to mathematical physicists, especially to those

working in quantum theory, and plausible but non-rigorous treatments are well known. Titchmarsh has succeeded in giving rigorous proofs and has put all quantum theorists heavily in his debt by his careful analysis of the complex and difficult questions of eigenfunction theory. His investigations of the expansion theory, the perturbation theory and the spectral theory are each of significance for the applied mathematician and, if not every proof can be described as short and simple, they are all admirably lucid and cogent.

G. TEMPLE

ELECTROCHEMISTRY

Electrochemistry

Principles and Applications. By Dr. Edmund C. Potter. Pp. xii+418. (London: Cleaver-Hume Press, Ltd., 1956.) 50*s*.

THE author states that his aim was to produce a concise introductory text for the technological student and the specialist electrochemist in which he would endeavour to reflect the modern emphasis on a more dynamic and mechanistic interpretation of electrolytic processes than, presumably, has hitherto been the case.

To write a book which would be of service to the beginner and the specialist alike would, indeed, be an accomplishment, and this the author has certainly not achieved. This book is more likely to be of use to the student who has already gained a grasp of the fundamentals of electrochemistry from the standard works on physical chemistry, and who wishes to acquaint himself with the industrial applications of the subject.

The mechanistic interpretation of electrolytic processes refers essentially to the interpretation of the dependence of hydrogen overpotential on current density. This has been included in text-books which have been published within the past twenty years.

Regarding the chapters dealing with fundamentals, the logical development and presentation often leave much to be desired: the English is not always good and the sentences are often involved and obscure. It is likely that the text was based on rather sketchy lecture notes, for it lacks much of that explanatory matter which a lecturer must expound but which the student may not then think it worth while to record.

The treatment of the Arrhenius, activity and Debye and Hückel theories is so cursory that a student would not be able to glean anything very substantial from it. Throughout, the term 'activity' is applied to ions, but the author does not indicate that the activity theory has only been satisfactorily applied to the electrolytes contained in 'cells without transport'. One such cell is described, but advantage was not taken to show how it might be used to determine experimentally the mean ionic activity of an electrolyte, for example, $a_{\pm HCl}$.

It is somewhat disturbing to read on p. 83 that "Nernst's original arbitrary zero of potential is that potential corresponding to the reversible equilibrium between hydrogen gas at one standard atmosphere pressure and hydrogen ions at unit activity". In 1900 Nernst stipulated that the electrode solution should contain hydrogen ions at unit concentration, and it was not until 1913 that G. N. Lewis advocated the substitution of activity for concentration. Dr. Potter does not explain that the activity of a single ion cannot be determined experimentally and that it is necessary to assume that, for example, for hydrochloric acid, $a_{\pm HCl}$, is equal to a_H. Instead, he merely states that "If the electrolyte used is hydrochloric acid a strength of approximately 1·2 *N* at 25° C. corresponds to unit activity of hydrogen ions", and he gives no clue how such a concentration has been experimentally ascertained.

The misuse of the terms 'strength', 'strong' and 'weak' in referring to solutions and current is to be deplored, for chemistry teachers have, for many years past, been deprecating the use of 'strength' as being synonymous with 'concentration', and 'current' (a rate) as being synonymous with 'electricity' (a quantity). Such phrases as "electric current flows" (p. 1) and "passage of current for 1 second" are particularly objectionable. Many of the conductometric titration curves depicted are defective, and especially that for phosphoric acid. In defining 'acids' and 'bases' in terms of the Lowry–Brønsted theory, 'hydrogen ions' should read 'protons'. The method of deriving expressions for electrode potentials can scarcely be adapted to the electrodes in a cell with transport.

Though by no means a complete survey of electrochemistry, the last half of the book is obviously that part of the subject with which the author is more intimately conversant and it brings together a good deal of useful material.

H. T. S. BRITTON

NEOMYCIN

Neomycin

Its Nature and Practical Application. Edited by Selman A. Waksman. Pp. x+412. (London: Baillière, Tindall and Cox, Ltd., 1958. Published for the Institute of Microbiology, Rutgers University.) 40*s*.

THE comprehensive screening programme to which the Actinomycetes, and in particular the members of the genus *Streptomyces*, have been subjected in various laboratories has provided many new antibiotics, a number of which are of value in chemotherapy. As a result of such a programme, Waksman and Lechevalier in 1949 announced the discovery of a new basic antibiotic which was named 'neomycin'. It was active against bacteria resistant to streptomycin, including tuberculosis organisms. It was thought that the new antibiotic might have all the desirable properties of streptomycin without its disadvantages, and that it could find an important place in tuberculosis therapy. Unfortunately, the high hopes at first entertained of neomycin were not fulfilled in practice, since clinical trials showed that when administered parenterally it had an irreversible injurious effect on the auditory system. Neomycin has, however, gradually established itself, in spite of its limitations, as a useful antibiotic and indeed the drug of choice in certain infections.

As commercially prepared, neomycin is a mixture of two isomeric compounds, neomycin *B* and neomycin *C*, in which the former predominates. Although the molecular structure of neomycin is not yet completely established, sufficient is known to place it as closely related to streptomycin, from which, however, it differs considerably in biological properties. It has a wider antibacterial spectrum than any other antibiotic, is rapid in action, shows little tendency to produce resistant organisms and rarely produces

hypersensitivity in the patient. Its disadvantages, the tendency to produce deafness and renal damage, are only evident when it is administered parenterally. It is one of the best antibiotics for local use and as an intestinal antiseptic by the oral route since it is not readily absorbed from the bowel.

The present work, appropriately under the editorship of Dr. Selman A. Waksman, offers a timely account of the information at present available, mainly from American sources, concerning the nature, microbiological aspects, pharmacological properties, and clinical and veterinary uses of neomycin. It is a compilation resulting from a co-operative effort to assemble between the covers of one volume the knowledge and experience of a number of investigators from different centres in the United States. Each chapter is written by one or more of these contributors, but due, no doubt, to careful planning and skilled editing there is little overlap, and the result is a balanced and most readable account of the present state of knowledge on the subject of neomycin, which could hitherto be assessed only by tedious search of the literature.

There is an excellent bibliography in which the references are sensibly collected together instead of being distributed at the end of each chapter (apart from a few supplementaries). Appendixes summarize the specific clinical uses of neomycin and list the preparations containing neomycin with their trade names and indications, compiled from information supplied by the various manufacturing companies, the names and addresses of which are given. This list may serve to correct the impression, which might otherwise be gained from reading the book, that streptomycin is of little interest outside the United States, since the number of foreign manufacturers cited is nearly twice that of firms located in the United States.

J. H. Birkinshaw

A THERMOPHILOUS SHRIMP FROM TUNISIA

Anatomie und Histo'ogie von *Thermosbaena mirabilis* Ein Beitrag zur Phylogenie der Reihe Pancarida (Thermosbaenacea). By Dr. Rolf Siewing. 1958. *Akademie der Wissenschaften und der Literatur. Abh. Math.-Naturw. Kl. Jahrgang* 1957, No. 7 : 197–270, 43 text-figs.

Dr. Siewing's recent admirable studies on the comparative morphology of the Crustacea Malacostraca have been widely acclaimed and no one is better qualified than he to elucidate the anatomy and histology of *Thermosbaena mirabilis* Monod. This minute crustacean, first described in 1924, is restricted in its distribution to a few baths, fed by hot springs, at El Hamma, Tunisia. In recent years three subterranean species, belonging to the closely related genus *Monodella*, have been found in Italy and Yugoslavia. These four species, measuring 1·5–4 mm. in length, are unique among Malacostraca in having a dorsal brood chamber formed by a temporary enlargement of the carapace. Opinion has varied as to the systematic position of the order Thermosbaenacea ; it is sometimes referred to the division Peracarida, or placed between the Peracarida and the Syncarida ; Sars united it with the Bathynellacea in a new group, the Anomostraca.

The material studied was collected by Dr. Anton Brunn in 1938. A detailed account, accompanied by admirably clear line drawings and microphotographs, is given of each system in turn—vascular, respiratory, nervous, digestive and reproductive. The reconstructions of the general anatomy of male and female are excellent, although an enlargement of the head end of Fig. 1, *A*, with its numerous guide lines, might with advantage have been included. Anatomical peculiarities are fewer than might have been expected. The oviduct opens in the usual Malacostracan position, at the base of the sixth thoracic limb, and there is no dorsally directed branch such as has been claimed for *Monodella* but not confirmed. Prof. Barker, who collected ovigerous females in September 1950, thinks that during oviposition *Thermosbaena* lies on its back so that the few large eggs drop into the brood chamber (personal communication). A pair of reduced, though possibly still functional, eyes has been found. Certain features appear to be adaptive, since they are paralleled in other minute cavernicolous or interstitial forms. For example, the extremely short heart is restricted to the first thoracic segment, where it is in intimate association with the respiratory carapace ; in the Bathynellacea, which have no carapace, it lies in the fourth thoracic segment.

An appraisal of the external morphology follows. At times the author is prolix ; for example, his interesting point about the segmentation of the thoracic limbs could have been made in a few paragraphs instead of in a few pages. In the second pair, which are set quite differently on the body and are apparently modified as gnathopods, the ischium has fused completely with the basis. In each of the succeeding limbs (peraeopods) it has almost completely fused with the merus. He concludes that the Thermosbaenacea, though closely related to, must be excluded from, the Peracarida. I am not entirely convinced of the necessity for the new division Pancarida, which is based chiefly on two characters : the structure of the maxillipeds and the dorsal brood chamber. The retention of two endites and the persistence, in some species of *Monodella*, of exopodite and/or endopodite in the maxilliped suggest, he says, a very primitive position for the Thermosbaenacea. This condition is widespread in the Decapoda and—the coxal endite apart—in Mysidacea. In *Monodella*, when both rami persist the maxilliped exhibits marked sexual dimorphism, the endopodite being modified to form a clasping organ in the adult male. As to the brood chamber : within the Copepoda certain parasitic forms have abandoned the normal method of carrying the eggs in favour of a carapacial brood chamber, and this has probably occurred independently more than once. Why then is the author so certain that something analogous could not have happened within the Peracarida (pp. 261–263) ? There might be some good reason why, in a minute animal adapted to an extreme biotope, a ventral marsupium composed of oostegites became impracticable (for example, the thoracic limbs might be too short). Owing to the high temperature to which *Thermosbaena* has become adapted (around 45° C.) its feeding and locomotor habits would have to be studied in the field. This could only be undertaken by a competent observer who, from previous experience with other minute crustaceans, knew exactly which methods to adopt.

Whether one accepts his conclusions or not, Dr. Siewing is to be congratulated on another excellent piece of research.

Isabella Gordon

Geophysics and the IGY

Proceedings of the Symposium at the Opening of the International Geophysical Year. Conducted by the United States National Committee for the International Geophysical Year, National Academy of Sciences, Washington, D.C., June 28–29, 1957. Edited by Hugh Odishaw and Stanley Ruttenberg. Pp. iv+210. (Geophysical Monograph No. 2.) (Washington, D.C.: American Geophysical Union of the National Academy of Sciences—National Research Council, 1958.) 8 dollars.

IN the whole of the programme for the International Geophysical Year nothing has captured the popular imagination more than the attempts to explore the upper atmosphere. It is likely that the layman does not entirely appreciate the scientific significance of all the *Sputniks* and 'Black Knights' that leave the surface of the Earth; but he is certainly made more aware of this aspect of the International Geophysical Year, via the writings in the press, than of any other; and, in a way, so it is for the geophysicist.

Almost three-quarters of the space available in this monograph on the International Geophysical Year is devoted to the physics of the upper atmosphere. Eighteen papers cover most aspects of this science from 'whistler' (audio-frequency radio waves arising from lightning discharges) studies to auroræ observations; and, of these, five concern the use of rockets and satellites in this high-atmosphere work. The papers in this section which are of most interest to the general scientists are probably those dealing with the observations to be made on the geomagnetic field. A detailed knowledge of the Earth's magnetic field is of practical importance in many forms of science and their related applications, from ionospheric and auroral physics to navigation and mineral exploration.

There are twelve papers in the more solid side of geophysics: that is, the lower atmosphere and the Earth itself. These deal with meteorological and oceanographic studies, the polar work that has been and will be performed, and with information concerning the shape, crust, and mantle of the Earth via gravitational and seismological measurements.

This book is the outcome of a symposium held last year before the main programme began. As such, of course, much of the information contained in it is conjectural and, at this stage, out of date. However, it is worthy of consideration by anyone interested in the doings of the International Geophysical Year.

D. Taylor Smith

Quantitative Organic Analysis

By James S. Fritz and George S. Hammond. Pp. xiv+303. (New York: John Wiley and Sons, Inc.; London: Chapman and Hall, Ltd., 1957.) 52*s*. net.

THE contents of this book are more accurately described by a subtitle ("The Use of Fundamental Principles for Solving Problems in Organic Analysis") than by its main title. The latter would lead one to expect the book to start with the usual description of elemental analysis, but this comes nearly half-way through. Instead, the opening chapter is an interesting discussion of the difficulties arising in the determination of carbonyl groups by oximation, which is taken as an illustration of the problems encountered in devising schemes for determining functional groups in general.

Other chapters deal with acid–base titrations in non-aqueous solvents, indirect acid–base methods, oxidative and reductive methods, manometric methods, metal ion complexes, kinetics in analysis, spectrophotometric methods, separations, and physical and polarographic methods. Finally, there are useful hints on solving new problems and details for certain standard procedures, such as determination of hydroxyl groups by acetylation, saponification of esters, and determination of sulphur, of unsaturation by bromination, and of peroxides.

This is not a text-book in the ordinary sense; rather does it discuss the underlying principles of various methods and their advantages and limitations. Although it mentions many applications, experimental details are usually rather too brief for practical purposes. For example, the ordinary 'combustion' method is described, but the diagram of the tube gives no indication of the dimensions in spite of the mention of the 'micro-scale'; and the statement that "Glucose has been determined by oxidation with iodine to gluconic acid" gives no hint of the effect of such factors as alkalinity, concentration and time. Also, under distillation, theoretical plates are mentioned but not defined. The section on chromatography is not up to date, and too cursory.

In spite of these few minor blemishes (and of the spelling of Tollens without his 's'), the book is well worth reading, for it is clearly written and gives a fascinating review of the great diversity in analytical methods now available to the organic chemist; moreover, it is very well produced.

A. D. Mitchell

Dynamic Programming

By Richard Bellman. Pp. xxv+342. (Princeton, N.J.: Princeton University Press; London: Oxford University Press, 1957.) 42*s*. net.

MANY will have had glimpses over recent years of the work of R. Bellman in the field of multi-stage decision processes. Gradually, a body of concepts and methods has emerged for treating the many real problems that arise in this field. The developments to date have been collected and presented in the book under review, which is a Rand Corporation Research Study. The author begins with a chapter entitled "A Multi-Stage Allocation Process". This explains the type of practical situation to which the theory of dynamic programming relates and introduces the reader to the methods of solution. The next chapter adds a stochastic element to the problem of decision on a multi-stage process. The mathematical technique is developed in the next two chapters, and then follow three chapters on inventory and bottleneck problems. A chapter on multi-stage games is followed by a final chapter on Markovian decision processes.

This book, while very well written, is definitely an advanced text for the mathematician who is interested in the development and application of mathematical methods in management science, including econometrics. It will not be understood by most managers, but this may well be the result of our present social organization rather than a reflexion upon the author. It is not, after all, possible to convey highly technical concepts in a language that everyone can understand. The present book is an excellent example of the way in which mathematics is finding new domains of application, and for the first time clears the way for logical analysis of some time-worn riddles faced by business men. L. S. Goddard

CYTOPLASM IN SEXUAL REPRODUCTION

By Prof. KENNETH MATHER, C.B.E., F.R.S., and Dr. JOHN L. JINKS

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GENETICAL studies have displayed the cytoplasm as playing but a minor part in the determination of differences among individual plants and animals produced by the sexual process. Evidently the cytoplasm handed on by a mother to her sexual offspring must be closely similar to that which she herself received from her parents in respect of any essential determinants of cell character and development. At the same time, there is every reason to believe that differentiation within an individual depends characteristically on change of the cytoplasm[1-3]. Thus, either the cell-lineage connecting the original zygotic cell of the individual with the egg it ultimately produces must be exceptional in involving no change of cytoplasmic type, or its cytoplasmic progression must come full circle within the individual, the end-point being the same as the beginning[1]. In higher animals there is no simple breeding test available for investigating the progression of a cell-lineage, of recognizing and separating progressive changes in nucleus and cytoplasm, by simple breeding; and even in higher plants this approach has yet to be put to successful use. Experimental intervention by the transfer of nuclei from cells at one stage to the cytoplasm of cells at another has been successfully practised and has yielded valuable results[4,5]. The implications of these findings are, however, as yet by no means fully clear[3], and in any event this technique is so demanding as to place sharp limitations on its use.

Micro-organisms present more tractable material, and the filamentous fungi in particular offer a certain, if limited, range of differentiation combined with the opportunity to propagate and breed from several types of cell in such a way that changes of the cytoplasm and nucleus can not only be detected but also distinguished from one another by reassociation of these cell parts. Thus, *Aspergillus glaucus*, for example, can readily be propagated in three ways: from the tips of the hyphæ, from the single-celled asexual spores termed conidia, and from the sexually produced ascospores. Propagation by any of these means produces an individual potentially capable of displaying the complete range of development, so that its capabilities may be fully observed and change from those of its parent recorded. Furthermore, the occurrence of anastomoses between hyphæ of different individuals, and the heterokaryosis to which this prospectively leads, allow of the bringing together of unlike cytoplasms as well as nuclei within a single individual, and of their sorting out and reassociation in both the conidia and the ascospores which that individual produces. Indeed, the conidia are especially valuable in distinguishing cytoplasmic from nuclear differences, for the nuclei of a heterokaryon normally sort out as units in the production of its conidia, without any of the genic recombination seen in ascospores. Thus, each kind of nucleus can be marked and followed unambiguously in conidial production by a single mutant gene.

Making use of these properties of the fungus, Jinks has been able to show not only that differentiation and ageing of the cells involve characteristic changes of the cytoplasm[6], but also that selection can be used to produce changes of character which spring wholly from alterations in cytoplasmic content[7]. Certain of these changes depend on differences among cells of like status, as where a sample of conidia from an individual produce offspring differing in their rates of growth so that selection may be practised among them. Others, however, appear to depend on cytoplasmic differences arising as part of the process of differentiation. Thus, if propagation is continued for several generations by conidia, the production of perithecia and ascospores gradually falls until the fungus at length loses its capacity for sexual reproduction and becomes imperfect[8]. If propagation is continually by hyphal tips, the capacity for sexual reproduction is similarly lost, but so later is that for the production of conidia also. This has also been observed in *Neurospora*[9]. Similar changes can, of course, come about by the change of nuclear genes within an individual; but these are spasmodic and show all the unpredictability of gene mutation, whereas the cytoplasmic alterations appear as characteristic of the normal processes of differentiation and development.

So far, these changes have shown us no more than the negative side of differentiation, for they all depend on the loss of a capacity which is no longer being used and which is therefore no longer of importance to the plant. If propagation is wholly by conidia, the production of sexual structures is of no significance and its loss of no disadvantage. Indeed, its retention could be actively disadvantageous by the profitless consumption of nutrients and metabolic materials capable of more useful deployment elsewhere, and it is thus no surprise to find conidial production rising at first as that of ascospores declines. But hyphæ must obviously continue to be produced, as they are a necessary link between parent and daughter conidia. Where, on the other hand, propagation is wholly by hyphæ, neither conidium nor ascospore takes any obvious role in the cycle, so that both may be lost with impunity, at least in the short term. It would thus appear that only capacities essential to the operative reproductive cycle are retained, the cytoplasm changing so as to lose the ability for sustaining types of development no longer of significance to the plant.

When, however, we turn to the sexual cycle a different picture emerges. With continued sexual propagation, the production of conidia is of no more importance than was that of ascospores with conidial propagation—yet conidia are never lost as a result of sexual propagation, nor indeed is the frequency of their production reduced in any way so far as can be seen. In contrast to the asexual, therefore, the sexual cycle maintains in the cytoplasm the full range of developmental capacities even where these are no longer of significance. Furthermore, this does not merely reflect an absence of cytoplasmic change in the cell lineage connecting parent ascospore and daughter, as we can see if we go back to lines the

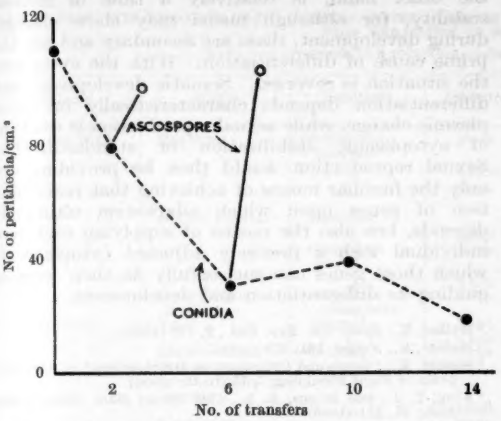

Fig. 1. The effects of propagation by conidia and by ascospores on the frequency of perithecial production in *Aspergillus glaucus*. Continued propagation by conidia (dotted line) results in a gradual decline of perithecial production. This is, however, immediately restored to its original level by a single propagation through an ascospore

sexual capacity of which has been reduced by conidial propagation. This reduction is gradual, but no matter how far it has gone, so long as any ascospore remains to be taken, the plant can be restored to complete, or virtually complete, sexuality by a single propagation through an ascospore (Fig. 1). Whether sexuality could be so restored to a plant which has completely lost its capacity for sexual reproduction is, of course, an unanswerable question since the necessary ascospore is not produced.

Thus, where a number of conidial propagations are interposed in the lineage between parent and daughter ascospores, the cytoplasm demonstrably changes in its determination of sexual capacity. It is, nevertheless, restored to its original state by the single propagation through the final ascospore. Evidently the process of sexual reproduction involves an active and positive re-adjustment of the cytoplasm, a 'spring-cleaning' or 're-tailoring', so that despite the alterations the standard basic cytoplasmic pattern is once again restored. There has been a cytoplasmic progression during the time of asexual propagation, but it is brought full circle during the process of sexual reproduction.

A further consequence of this re-standardizing effect of sexual reproduction on the cytoplasm is seen when the variation between individuals arising from ascospores is compared with that between individuals from the conidia of the same parent plant. Where the parent is a heterokaryon, genic segregation and recombination will occur in its sexual offspring. Their variation may thus be greater than that of conidial offspring, in the production of which only sorting-out of whole nuclei can occur. But if the parent is a homokaryon, carrying only one genetical type of nucleus, genically determined variation must be absent from all the offspring, whether sexual or asexual, and can indeed be shown to be absent by suitable tests. The variation is then found to be much greater among the conidial than among the sexual progeny (Fig. 2). In other words, the cytoplasm is more variable between the conidia than between the ascospores. Again we are seeing the standardizing effect of sexual reproduction on the cytoplasm.

Comparison of the effects of propagation by ascospores and conidia indicates an active re-standardization of the cytoplasm during the sexual process, though, of course, it does not tell us precisely where in the sexual cycle this occurs; further observation will be needed for that. Comparison of the effects of propagation by conidia and hyphal tips suggests that some readjustment of the cytoplasm may take place during conidial production, though it must obviously be less comprehensive and precise than that occurring in the sexual cycle. Continued propagation by hyphal tips results first in the decrease and loss of ascospore production, then in the decrease and loss of conidial production, and finally in a progressive difficulty even of the hyphæ to maintain full growth. So long, however, as conidia are produced the intervention of a conidial propagation will at least partly restore the capacity for conidial production, and it will improve hyphal growth itself. Furthermore, certain cytoplasmic adaptations, as for example to tolerance of mercuric chloride in the medium, though regularly transmitted via the hyphæ, are passed on much less regularly through conidia[10], and not at all through ascospores. Thus, the process of cytoplasmic re-standardization, though shown most characteristically and precisely by the sexual cycle, may not be confined to it.

Should sexual reproduction always involve a standardizing or cleaning of the cytoplasm, as it appears to do in *Aspergillus glaucus*, the cause of the cytoplasm's apparent inertness in hereditary transmission, as displayed by genetical experiments, would be clear. There is, however, as yet, little evidence from higher plants and animals to show whether such a process occurs or not. With some exceptions, chiefly in the Leguminoseae, plant virus infections are not transmitted through the seed. This suggests that the cytoplasm may be actively readjusted during egg-production, though the evidence cannot be regarded as compelling before it has been shown that the virus does actually enter the cell-lineages of the growing points from which the eggs are ultimately produced. We know also that the side-shoots of some plants show a type of growth different from that of the main shoot when both are taken as cuttings[11]. It would be of great interest to know how the two types behave in subsequent

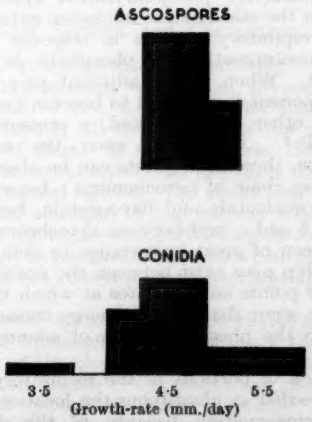

Fig. 2. Histograms to show the frequencies of colonies with different rates of growth among (above) the sexual and (below) the conidial progeny of a homokaryon of *Aspergillus glaucus*. The conidial progeny are more variable

breeding. In this way it would be possible to show first whether the difference itself involved nuclear change, and given that it did not, whether the cytoplasmic difference was abolished during sexual reproduction. By analogy with *Aspergillus*, we should expect the sexual offspring of both types of cutting to be like the original plant from which the cuttings were taken. There is evidence that the cytoplasm of parthenogenetic eggs in Rotifers can change with the age of the mother[12], and even that the cytoplasm can vary in the eggs of *Drosophila melanogaster*[13]; but the variation in the flies appears to be trivial, and in any event a relation to ageing does not preclude the occurrence of a process such as that detected in the fungus, since ageing of the egg could follow on a standardization of the cytoplasm during its initiation. In man, fraternal twins may be quantitatively more alike at birth than identicals. This can be interpreted as indicating that the cytoplasms of separately produced eggs are more alike than those of two totipotent cells produced from the division of a single egg[14], as might be expected if a process of cytoplasmic re-standardization occurs during human sexual reproduction.

Where, as in *Aspergillus*, a process of cytoplasmic standardization occurs as a regular feature of sexual reproduction, a remarkable comparison appears between nucleus and cytoplasm. Sexual reproduction is the time of nuclear variation, springing from recombination of the genes. Somatic development, on the other hand, is relatively a time of nuclear stability, for although nuclei may show changes during development, these are secondary and not the prime cause of differentiation. With the cytoplasm the situation is reversed. Somatic development and differentiation depends characteristically on cytoplasmic change, while sexual reproduction is the time of cytoplasmic stabilization or standardization. Sexual reproduction would then be providing not only the familiar means of achieving that redistribution of genes upon which adaptation ultimately depends, but also the means of supplying each new individual with a precisely adjusted cytoplasm in which those genes can successfully do their work of guiding its differentiation and development.

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LOCALIZATION OF INTERACTION SITES IN MULTI-COMPONENT TRANSFER SYSTEMS: THEOREMS DERIVED FROM ANALOGUES

By Prof. BRITTON CHANCE, WILLIAM HOLMES and JOSEPH HIGGINS

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AND

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A STRIKING phenomenon of electron transfer in oxidative phosphorylation systems is the change in the steady-state oxidation-reduction levels of the respiratory carriers in response to changes in the concentration of phosphate or phosphate acceptor[1]. When, in an adjacent pair of carriers, one component is observed to become more oxidized and the other more reduced, a crossover point is identified[2-3]. Depending upon the experimental conditions, three such points can be observed in the respiratory chain of mitochondria: between reduced pyridine nucleotide and flavoprotein, between cytochromes *b* and *c*, and between cytochromes *c* and *a*[1]. It has been of great importance to determine what relationship may exist between the position of these crossover points and the sites at which the chemical reactions occur that lead to energy conservation and finally to the phosphorylation of adenosine diphosphate.

Isolation of portions of the respiratory chain has not succeeded in identifying the location of sites of energy conservation because of the difficulty of isolating components of the chain[4], the loss of phosphorylative activity upon isolation[5], and the demonstrated changes in the pathways of electron transfer that may arise upon chemical fractionation of the components[6-8]. Thus, fractionation and reconstitution cannot yet be used for final proof of conclusions that are drawn from physical-chemical studies of the phosphorylative process.

At present, it is necessary to deduce the location of sites of energy conservation from a consideration of the response of a chain of electron-transferring enzymes to the presence or absence of an inhibitor that can act simultaneously at several sites in the chain. Such a multi-component system is difficult to comprehend without the derivation of general theorems concerning the response of such systems by mathematical procedures and by observations of the response of model systems or of analogue-computer representations of various mechanisms.

All phenomena associated with cross-over behaviour were first observed in direct studies of the respiratory enzymes of mitochondria, during which it became apparent that certain rules permitting the localization of phosphorylation sites might apply. The cross-over

Table 1. Cross-over Behaviour of the Chemical System: Changes of Steady-state Level caused by a Decrease of Flux resulting from Exhaustion of Adenosine Diphosphate

Key: − corresponds to a reduction, + to an oxidation, 0 to no observable change

Material	Source	Substrate	Inhibitor	Temp. (° C.)	Reference	S	RPN	fp	b	c_1	c	a	a_1	O_2
Mitochondria	Rat liver	β-Hydroxy-butyrate	None	26	1	−	−	−	−	+	+			
	,,	,,	Azide	2	12	−	−	−	+	+	+			
	,,	,,	None	26	Exp. 377	−	+	−	−	+				
	,,	,,	,,	38	15	−	+	−						
	Guinea pig liver	Succinate	,,	26	Exp. 376b	−	−	−	−	+				
	,,	,,	Azide	,,	Exp. 384	−	+	0	+	+				
	,,	Glutamate	None	,,	1, Exp. 372	−	−	−	+	+	+			
	,,	,,	,,	,,	Exp. 376b	−	+	−	+	+				
	,,	,,	Azide	,,	Exp. 384	−	+	0	+	+	+			
	Locust flight muscle	α-Glycerol phosphate	None	20	16	−	−	−	−	+				
Intact cells	Yeast	Ethanol	None	26	17	−	−	−	−	+	+			
	,,	,,	Azide	,,	Exp. 543			−	+	+				
	Ehrlich ascites tumour	Glucose	None	,,	18	−		−	−	+				
	Frog sartorius muscle	,,	,,	,,	19, 20	−		−	−	+				
	,,	,,	Azide	,,	19			0	+	+	+			
	Toad heart muscle	,,	None	,,	21	−	−	−	+	+				

behaviour of the chemical system has stimulated a number of studies to demonstrate generalities relating interaction sites to cross-over behaviour. The first study was made with an electronic analogue computer representative of the chemical system that operates in accordance with the law of mass action[2] and has led to some general rules summarized here. These rules, in their more elementary form[2], stimulated a mathematical approach involving inequalities applicable to many other types of mechanism[3]; this approach is presented in detail in another paper[9]. Holton[10,11] has recently used a current-flow network to demonstrate that interaction in physical as well as chemical systems can be located by the cross-over generalities given here. The more general applicability of these ideas justifies their detailed description.

Responses of the Respiratory Chain

Since spectroscopic observations of the response to adenosine diphosphate of isolated mitochondria and of mitochondria in intact cells and tissues are now extensive, a summary is appropriate (Table 1). In the course of these studies it was found that the appearance of a cross-over point depends, in specific cases, upon the balance of dehydrogenase and oxidase activities and, in general, upon relative values of reaction velocity constants for the interaction of the components of the respiratory chain. These velocity constants are affected by many factors, particularly the source of the mitochondria, the nature of the substrate, the presence of inhibitors, and the temperature. So that the data will conform with the results obtained from analogues discussed in the following paragraph, the responses are tabulated for decreases of flux caused by the exhaustion of adenosine diphosphate. A minus sign corresponds to a reduction, a plus sign to an oxidation, and a zero to no observable change (presumably the result of a balance of oxidizing and reducing tendencies). From the data of Table 1, we draw the following generalizations. (1) Only minus signs are observed for reduced pyridine nucleotide, and only plus signs are observed for cytochromes a and a_3 in isolated mitochondria. (2) The other components may show minuses, pluses, or zeros, depending upon the conditions. (3) Cross-over points (− to +, going from substrate to oxygen (see below)) localize interaction sites between reduced pyridine nucleotide and flavoprotein, b and $c + c_1$, and $c + c_1$ and a. Reversed cross-over points (+ to − (see below)) are found between flavoprotein and b. Since the resolution of the spectroscopic method is insufficient to distinguish accurately between cytochromes c and c_1, it is not known whether another cross-over point lies between these two components; however, thermodynamic considerations make this unlikely. In toad heart muscle, some preliminary evidence for a cross-over point between a_3 and oxygen has been obtained, but this is not supported by studies of the isolated toad heart mitochondria (Ramirez, J., personal communication). The three cross-over points localized thus far are consistent with the P to O value of the respiratory chain of approximately 3, although additional sites of phosphorylation are not ruled out by any existing data.

The data for intact cells, although less complete than those obtained for isolated mitochondria because of the inherent difficulties in controlling the type of substrate employed, show two of the three cross-over points observed in the isolated systems. This type of study should afford a basis for conclusions on the similarity between the phosphorylation efficiency of intact cells and that of isolated mitochondria.

Model Systems and Computers

In general, model systems are constructed for the purpose of representing a complex process on a convenient scale of size, time, etc. The model may represent accurately all the features of the original, or just some of them. In the latter case, the model may also demonstrate artefactual responses that must be carefully guarded against. The model may be constructed as an analogue of the process itself: for example, as a chemical system with simpler reactions or as an electric or hydraulic analogue where chemical concentrations are represented by voltages or liquid volume.

A more general approach is the computer solution of differential equations which represent the basic physical laws of the chemical system. This method has been used in studies of the respiratory chain and its associated phosphorylation reactions[2]. This model is based upon the assumption that the law of mass action applies to the reactions of the components of the respiratory chain[2]. On the basis of

Table 2. Cross-over Behaviour of Electron-transfer System caused by Decreases of Flux in response to Changes in Interaction at Three Sites

Enzyme concentration 10^{-6} *M*. Reaction velocity constants are specified in equations or in table in units of μmolar^{-1} sec.$^{-1}$. k_2, k_4, and k_6 are decreased from 0·9 to 0·09 μmolar^{-1} sec.$^{-1}$ to give the steady state concentration changes shown in units of 10^{-6} molar. Values for k_3 and k_5 are given in the table

$$S \xrightarrow{k_1 = 0{\cdot}9} \mathrm{RPN} \xrightarrow{k_2 = 0{\cdot}9} b \xrightarrow{k_3} c \xrightarrow{k_7 = 0{\cdot}9} a \xrightarrow{k_8 = 0{\cdot}9} a_3 \xrightarrow{k_9} O_2$$

(k_2 to A; k_4 to A; k_6 to A)

k_3	k_5					
0·9	0·10	− 26	− 47	− 30	+ 23	+ 44
0·9	0·07	− 12	− 5	+ 18	+ 85	+ 49
0·9	0·04	− 4	+ 14	+ 33	+ 85	+ 25
0·2	0·5	− 10	+ 17	− 12	+ 12	+ 7

this single assumption, one may make an accurate mathematical model of the complex sequence of reactions. The equations for electron transfer through the eight bimolecular steps are written in a shorthand notation in which each chemical equation consists of the reactions above one another, the velocity constant and reaction products being indicated by following the arrows[8]:

$$O_2 \xrightarrow{k_1} a_3''' \xrightarrow{k_8} a''' \xrightarrow{k_7} c''' \xrightarrow{k_6} c_1''' \xrightarrow{k_5} b''' \xrightarrow{k_{11}} fp \xrightarrow{k_{12}} \mathrm{RPN} \xrightarrow{k_{13}} \mathrm{AcAc}$$

$$O_2 \xleftarrow{k_1} a_3'' \xleftarrow{k_8} a'' \xleftarrow{k_7} c'' \xleftarrow{k_6} c_1'' \xleftarrow{k_5} b'' \xleftarrow{k_{11}} rfp \xleftarrow{k_{12}} \mathrm{RPN} \xleftarrow{k_{13}} \mathrm{BOH}$$

Additional reactions causing inhibition of the bimolecular processes are represented by the combination of an inhibitory substance (I) with one of the reactants, so that the concentration of the reactant available for electron transfer is diminished. Physical argument readily indicates that an inhibitor acting at a single point by combining, for example, with the reduced form of a respiratory component would cause a decrease in the concentration of the oxidized form of those components on the substrate side of the interaction site, and an increase in the concentration of the oxidized form of those components on the oxygen side of the interaction site. Thus when there is only one interaction, the site is invariably identified by a − to + cross-over.

Table 2 gives values for the changes of the steady-state oxidation-level of the cytochrome components in response to a decrease in the velocity constant for interaction with the substance A. This simulates the exhaustion of added adenosine diphosphate. In this calculation we have summed the concentrations of the active and inactive forms of the intermediate, since in the chemical system these are apparently spectroscopically indistinguishable[8]. The first row of data simulates conditions for the cytochrome chain where the cross-over point is found between cytochromes *c* and *a*. A decrease in the velocity constant for the oxygen reaction (experimentally caused by the addition of azide[12]) can move, in successive steps, the cross-over point to between components *b* and *c* or between components reduced pyridine nucleotide and *b* as indicated in the second and third rows. These analogue-computer data verify the method which we have used experimentally for showing that three sites of interaction exist in the respiratory chain of mitochondria. In the fourth row the velocity constant between cytochromes *b* and *c* is diminished and a 'reversed cross-over' is obtained. The − to + cross-over points between reduced pyridine nucleotide and *b* and between *c* and *a* are, however, valid indications of interaction sites.

In a five-component system, together with further data not included here on a seven-component system, this analogue computer representation of three sites of interaction affords a basis for restatement of the cross-over theorem in a more useful form than that given previously. We will state the theorems that apply to a system involving flux from a substrate to oxygen through a series of carriers which exist in oxidized and reduced forms. The reduction of components is denoted by − and their oxidation by +. For interactions that cause decreases of flux, we can state: (1) an interaction site lies between a − to + change in the sequence from substrate to oxygen; (2) components between oxygen and the first site will always show + changes, while those between substrate and the last site will always show − changes; (3) a cross-over point near the oxygen end of the chain can be shifted to the next site of interaction by a decrease of activity in the oxidase portion of the chain and vice versa; (4) a + to − change (reversed cross-over) does not identify an interaction site.

These theorems appear to apply to many other types of systems, for which the term 'substrate' can be replaced by the term 'source' (source of flux) and 'oxygen' by 'sink' (a sink for the flux). In this case, 'reduction' (−) implies an accumulation of the source material and 'oxidation' (+) a depletion of the source material. In the case of a semi-conductor, the substrate would be represented by electrons and the oxygen by holes.

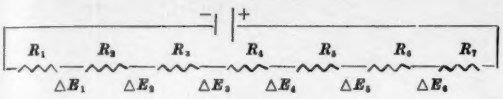
The demonstrability of cross-over points for all sites of interaction depends on a number of factors, the most important of which are the intensity of the interactions and their effect upon the flux through the system. Another factor to be considered is the steady-state level; components which are nearly fully oxidized or reduced may show changes that are difficult to measure, since a component that is highly oxidized will be insensitive to an oxidizing interaction and vice versa. In such cases, inhibitors are useful to alter steady-state levels and thus to increase the demonstrability of cross-over points. Inhibitors also shift the cross-over point from site to site along the chain and thereby allow successive demonstrations of the interaction sites of the chain.

Current- and Fluid-flow Models

Holton[10] and, more recently, Lundegårdh[13] have respectively proposed current- and fluid-flow models for the electron transfer system, and the former has made a detailed study of the response of the current-flow model to changes of resistance[10] to determine whether it simulates cross-over behaviour. His corrected results[11] agree with the generalization obtained with the analogue computer and show the movement of the cross-over point (− to + change) with decreases of flux illustrated in Table 3 together with a + to − change also indicated by the last row of Table 3. Such a reversed cross-over does not indicate a site of interaction (see below).

One of us (C.M.C.) has made a complete analysis of a current-flow model containing seven resistors in which R_2, R_4, and R_5 may be increased (see Table 3). It is found that ΔE_1 is always negative and ΔE_5 and ΔE_6 are always positive. We define, in accordance with Table 1, a cross-over point as a − to + change

Table 3. Cross-over Behaviour of a Current-Flow Model, Illustrating the Conceivable Responses to Decreases of Current Flow caused by Increases of Resistance at Three Sites—R_2, R_4, and R_6



	ΔE_1	ΔE_2	ΔE_3	ΔE_4	ΔE_5	ΔE_6
a	−	−	−	+	+	+
b	−	−	−	+	+	+
c	−	−	+	+	+	+
d	−	+	+	+	+	+
e	−	+	+	−	+	+
f	−	+	−	−	+	+
g	−	+	−	+	+	+
h	−	−	+	−	+	+

proceeding from e_1 to e_8 in response to a decrease of flux or current flow. If one sets down all the conceivable combinations of signs for ΔE_2, ΔE_3, and ΔE_4 an analysis of the circuit shows rows *a*, *b*, *d*, *e*, *f*, and *g* to be realizable with appropriate changes of flux. Thus all three sites of interaction can be readily identified by the − to + cross-over. Rows *c* and *h* appear to identify false sites of inhibition, but the analysis further shows that no combination of resistance changes can possibly produce potential changes leading to rows *c* and *h*. In summary, a − to + change associated with a decrease of flux identifies one of the sites at which interaction has occurred.

Neither the current- nor the fluid-flow model represents the bimolecular interaction of electron transfer in the chemical system. The reaction of soluble cytochrome *c* with the cytochrome *a* component of a solubilized cytochrome is bimolecular :

$$c'' + a''' \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} c''' + a'' \qquad (1)$$

This reaction holds for lower cytochrome *c* concentrations, although the reaction at high cytochrome concentrations is complicated by the fact that this substance is inhibitory[14]. No reversibility of the reaction has been demonstrated, that is, $k_1 >> k_{-1}$. The same rate law holds to a reasonable accuracy[22] for systems in which the carriers are bound to structures which, in the case of the mitochondria, are probably the cristae. The current-flow model (Holton, F. A., personal communication) appears to represent equation 1, but k_{-1} must be equal to k_1, a condition clearly not true for the chemical system. A more general analysis shows that the current-flow system represents, in addition to the reversible system of equal forward and reverse velocity constants, an irreversible system with a rate constant equal to the reciprocal of the sum of all the resistances.

The kinetic response of the current- and fluid-flow models is not representative of equation 1. While Holton makes no claim that the current-flow model represents chemical kinetics, Lundegårdh does so for the fluid-flow model. The chemical equation for the next step in the reaction is :

$$a'' + a_3''' \underset{}{\overset{k_3}{\longrightarrow}} a_3'' + a''' \qquad (2)$$

From equations 1 and 2, the differential equation for $k_{-1} = 0$ has two product terms :

$$-\frac{da'''}{dt} = k_1 c'' a''' - k_3 a'' a_3''' \qquad (3)$$

As illustrated by this example, and for any mechanism in general, the rates of reactions in a cytochrome system depend on two chemicals, since oxidation-reduction reactions are involved. The systems of Lundegårdh and Holton depend on the analogue of only one chemical, and thus differ in a most fundamental way.

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WATER RESOURCES AND WATER NEEDS IN SCOTLAND

A SYMPOSIUM on "Water Resources and Water Needs in Scotland" was held by Section E (Geography) of the British Association on September 1. The following papers were read : "The Need for River Flow Studies", by R. Maclagan Gorrie (Royal Scottish Geographical Society) ; "Measuring River Flow and Estimating Floods", by P. O. Wolf (University of London) ; "Evaporation and Land Use in Water Conservation Studies", by F. H. W. Green (Nature Conservancy) ; "Hydro-electric Development in Scotland", by P. L. Aitken (North of Scotland Hydro-electric Board) ; and "Industry in relation to Water Supply", by L. B. Aitken (Scottish Council Development and Industry).

The abundance of pure water has in the past been a major influence in the siting of Scottish developments such as the early linen and cotton mills, paper making, brewing and distilling. It also fixed the sites for aluminium production, and, more recently, for the North of Scotland Hydro-electric Board's sites in Perthshire, Argyll and Ross. The new atomic power stations and chemical factories make very big demands on water supply, so requests for 5 million gallons a day are now common.

The demand for electricity in Scotland is growing by 7 per cent per annum, so that new power stations have to be planned to meet a doubling of output every ten years. The base load which is maintained

over the whole twenty-four hours can best be met by steam or nuclear stations, but the fluctuations above this minimum are a different matter, and can best be met from steam power or by hydro-electric stations. The peak load may be for only a few hours in each day but will cost three times as much as the base load to produce. Hydro-electric stations of the conventional or of the pumped-storage type are best suited to produce the peak load and can do so at less cost.

Scottish river supplies are thus so valuable that every effort must be made to safeguard them, and the use of this basic resource must be planned. The behaviour of rivers can best be judged from stream-gauge records, but the amount of water reaching a stream-bed is an uncertain fraction of the rain which has fallen within its catchment. To get an accurate picture of the value of a river we must therefore have a knowledge of the rainfall, not only in total but also in intensity, and with enough rain-gauges to provide a grid which will show the pattern of local storms.

Stream-gauging has lagged seriously behind other countries; in Scotland it was left to private initiative, the pioneer being Capt. W. N. MacClean, who, at his own expense, undertook gauging from 1912 onwards on the Rivers Garry, Moriston, Ness, Lochy and Dee. The data he recorded are of great value, but unfortunately this was not appreciated when he offered them to various Scottish bodies; these records are now being used by the London School of Economics for the training of hydrologists.

The "Surface Water Year Book", now issued officially, covers from 1945 onwards. The Scottish mainland has forty-two major catchments, out of which rainfall–run-off data are presented for sixteen of them or their tributaries. Unfortunately, in the case of two of our most important rivers, the Tay and the Spey, the rainfall data are considered by the Meteorological Office to be inadequate to present rainfall in detail, and the flow of both is affected by transfers of water between the two across their common watershed for hydro-electric supply. Excluding the Tay and Spey, ten catchments of more than a hundred square miles and seven catchments of less than a hundred square miles are recorded, so that their rainfall–run-off ratios can be determined. The readings for all of them for a number of years give a straight-line graph showing a ratio of run-off (A) to rainfall (R) of $A = 7R/8 - 8$ (in inches). This differs radically from that given for the Thames basin by E. C. Bilham, in which $A = 0{\cdot}57R - 6{\cdot}05$ (in inches). This confirms that their behaviour is radically different, as must be expected considering the greater altitude and heavier rainfall of the Scottish Highlands and the very large area of peat which covers them.

Evaporation (including the transpiration from plants) is the largest single item in accounting for the difference between rainfall and run-off. In assessing this loss, two fundamentals have to be separated, namely, the energy causing the evaporation and the water available for the process. By guarantee that water supply is never a limiting factor, we arrive at a figure of 'potential evaporation' which can be considered as a climatic factor comparable with, say, rainfall or air temperature, but to make valid comparisons there must be more or less arbitrarily defined standard conditions.

A grass sward free of obstructions and conforming to the ordinary requirements for a meteorological reporting station has been used by F. H. W. Green,

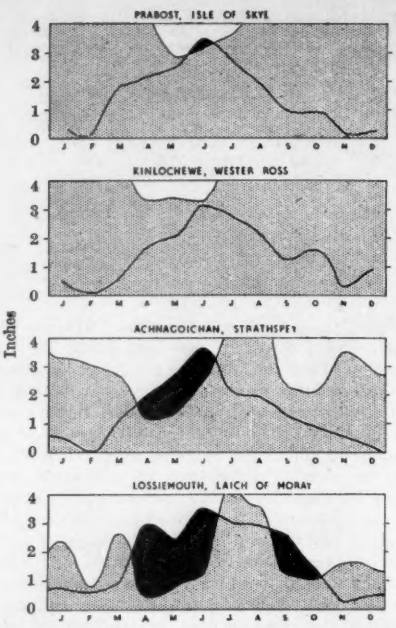


Fig. 1. ——, Rainfall; ——, potential evaporation; ■, excess of potential evaporation over rainfall, all for 1957

of the Nature Conservancy staff, in producing valuable contrasts in four Highland readings, as shown in Fig. 1. Kinlochewe is in the maximum rainfall belt, immediately to the west of the main watershed; Prabost in Skye is to the west of this, and Lossiemouth well to the east of it. Achnagoichan in Speyside is intermediate in an intermontane basin but at an elevation of 1,000 ft. As the effect of alternative land uses upon the available water supply is the ultimate objective, steps are now being taken to determine the potential evaporation in the centre of a large homogeneous area of spruce.

A historical analysis of damaging floods so far recorded in Scotland gives the impression that floods and damage occur more frequently at present than they did in the past. When one attempts to analyse this trend in terms of changing land use and run-off conditions, there is already apparent a widespread deterioration in the condition of our moorland peat as a ground cover. This has been brought about by the moor burning, which has increased in many districts as an answer to the coarsening of the grasses following upon the use of sheep only over a long period of years. When robbed of its protective cover by fire, peat behaves in the same way as any other eroding soil; in the first heavy rain after the fire it forms a surface slime which seals off the top, thus preventing normal penetration of the rain through the underlying layers of soil and rock to join the water-table. Instead, it all goes off in surface run-off which, of course, increases the 'flashiness' of flood peaks.

Other changes in land conditions which contribute to damaging flood peaks are the increased bank erosion along many of our highland streams, the use of heavy drainage ploughs on sheep-runs and afforestation areas, and the commercial 'skinning' of peat from the bogs for commercial purposes. Flood intensities are clearly a function of the intensities of

rainfall and of the catchment characteristics, including the limits of retention capacity as influenced by land use and misuse. The hydrological estimate of 'maximum possible floods' or 'probable maximum floods' starts, however, with studies of storm maxima, either by transposing the worst recorded storm for the region on to the catchment in question, or by building up from other meteorological data.

Since the Reservoir (Safety Provisions) Act, 1930, has laid the responsibility for checking the safety of dams upon a panel of engineers, the technique advocated in the Institution of Civil Engineers' Interim Report, 1933, is to plot the peak discharges for all the recorded rivers in Britain in cusecs per thousand acres of catchment for the actual catchment area ; the curve thus produced has been of the greatest value as a guide in experienced hands. A revision of this report is now in preparation and will include flood data collected between 1933 and 1957, and this will be a further step in helping the designers of dams and also those wrestling with flood forecasting.

Local authorities are continually trying to attract new industry to their area, but in many cases do not know what their water resource amounts to. Demands of 5 million gallons a day, or even 20 million, are now met with ; but the number of sites which can cater for such demands is limited, and when the few still to be exploited in England are used up, there will remain only the Scottish Highlands. Unfortunately, we do not know the potential of many Scottish rivers and it is imperative that more data and more reliable data should be collected. Guesses are not good enough ; an industrialist will site his factory where he knows there is enough water, and not on another more desirable site where there might be enough.

In the past it has not been the duty of a local authority to measure river flows. It is a highly specialized job and an expensive one if carried out according to the high standards of the Land Drainage Division of the Department of Agriculture for Scotland. The programme of the Division is being greatly extended, but even so, only a fraction of Scottish rivers is now being measured. Under the river purification boards now appointed for eight lowland rivers in Scotland, minimum or nadir flows are being measured, as this is the crucial figure needed to determine permissible pollution. Other county officers, including planners, road engineers and police, want data for safety precautions and flood warning systems, and are adopting various types of gauging less expensive than the Land Drainage Division's specification.

The Royal Scottish Geographical Society has recently established a standing committee for river flow studies and is defraying the expenses of meetings ; the group includes the four university geography departments, civil engineers, land drainage and water use specialists, meteorologists and land use planners. The main tasks are to collate flood records, review any research work and encourage fresh efforts in research and survey of river behaviour, and ensure publication of suitable material ; to advise local authorities when called upon ; and where warranted, to agitate for action on flood control ; to arrange the formation of 'flying squads' for immediate reconnaissance of floods as they occur ; and to encourage longer-term studies bearing upon evapo-transpiration and the ultimate demands on existing water supplies. So far, the committee's main function has been to provide a platform for discussion ; the organization is a very long way behind its Danish counterpart, the Hydrology Section of the Danish Heath Society at Slagelse. Hitherto, efforts to get funds have failed and the programme is crippled because of this.

The measurement of the flow of water, starting with surface run-off and finishing in the sea, can properly be considered the sphere of geographers skilled in hydrometry ; the basic knowledge must needs include the ecology and geology of the catchment as well as the engineering of a gauging site, the best equipment for the given conditions, and the drill for using the instruments properly. Too much of current press writings about floods and water resources shows a superficial approach. It is hoped that this may be counteracted to some extent by getting an increasing number of geographers using a scientific approach and in close collaboration with the engineers to undertake hydrometric work as well as climatological stations, and so build up a fuller knowledge of the nation's water resource.

R. Maclagan Gorrie

NUCLEAR POWER STATIONS

WORLD PLANS AND EXPERIENCE

At the first Geneva Conference on the Peaceful Uses of Atomic Energy in 1955 only the U.S.S.R. could claim to have a nuclear power station in operation, and that on little more than an experimental scale. Producing 6,000 kW., it was generally considered, from the description provided, to be no more than a demonstration of feasibility. The building of Calder Hall was well advanced, but it was not in operation and its economy and reliability were still to be demonstrated. The United States, with ample and cheap supplies of fossil fuels, felt less urgency for the development of nuclear power stations and greater difficulty in achieving economic parity with conventional stations at home.

The three years between the first and second Conferences at Geneva have seen very great advances in this field in many countries. The feasibility of nuclear power stations is no longer in question and discussion now centres on the type of reactor to be used, the date by which it will provide electricity as cheaply as conventional stations, in particular national economies, and the rate at which nuclear power stations can be built. In 1955, only the United Kingdom had a national nuclear power programme, and it is significant of the rate of advancement in this period that within two years this programme had been substantially increased, based on the development potential revealed by the design of the first stations. At the second Conference recently concluded, a number of countries presented papers setting out their plans for the construction of nuclear power stations, not as vague and distant hopes, but

covering generally the next decade and based on careful assessments of demands for electricity, supplies and costs of fossil fuel, the economics of reactor systems and the availability of finance and technological man-power.

Between those countries where fossil fuel or hydro reserves are at present adequate, such as the United States and Sweden, and those which urgently require a new source of energy, such as Japan and Italy, there are a number of differences which have led to two main approaches. The former group tend to have wide ranging programmes of prototype demonstration reactors, whereas the latter concentrate on systems already developed in other countries. Western Europe, through the agency of Euratom, may be in a special position, combining concentration by individual countries with diversity over the whole Euratom area.

Running through most of the papers presenting national programmes is the determination to achieve ultimate independence, at least in the design and construction of the stations, although several countries will clearly have to rely on imports of uranium and thorium for fuel. At the same time, there is recognition of the heavy demands on scientific and technological man-power, as well as on industrial capacity, which the development of a reactor system to a commercial scale makes, and the realization that in the early stages there must be some degree of dependence on other countries.

For those countries which have accepted the need to import their first nuclear power stations, the choice virtually rests between two systems: the graphite-moderated, gas-cooled reactor developed from Calder Hall, and the water-cooled and moderated systems, in the form of the pressurized water reactor at Shippingport and the boiling-water reactor nearing completion at Dresden in the United States.

No very clear picture as to the relative merits of cost of power in these two types of station emerged. The gas-cooled reactor has high capital cost, which may be reduced if it is built for the large outputs of which it is capable, and low fuel costs. The boiling-water reactor, particularly, appears to have low capital costs but these are associated with higher fuel charges. Thus, an economic comparison is very dependent upon the size of station to be built and the rate of interest chargeable to the capital investment. The fact that the former uses natural uranium, thereby eliminating the need for a diffusion plant or the importation of enriched fuel, offers the prospect of earlier national independence.

Although the number of power reactor systems already built and operating is limited, the range will clearly be extended in the near future, with the United States and the U.S.S.R. leading the way in diversity. The United States, having adequate conventional fuel resources, has been concerned chiefly with the development of reactors for submarine propulsion, and in the field of electricity generation has encouraged the construction of power demonstration reactors, of relatively small output, primarily with a view to export. The availability of comparatively cheap enriched uranium has been invaluable in permitting the study of small reactors of various types, but it is noteworthy that the U.S. Atomic Energy Commission has also turned its attention recently to the gas-cooled, graphite-moderated system.

The United Kingdom, while pursuing its policy of concentrating its main effort on developments of Calder Hall to provide large units for the home market and introducing modifications such as ceramic fuel and beryllium cans to permit higher operating temperatures and consequent greater efficiency, is considering alternatives such as the replacement of graphite by heavy water as the moderator. That such alternatives must satisfy the criterion of offering economic advantages over developments of the Calder Hall type is clear from the announcement of the abandonment of work on the sodium-cooled graphite-moderated system, which was adjudged to offer no economic inducement. Both the United Kingdom and the United States are devoting considerable effort to the longer-term project of a fast fission reactor, and the advantage of the positive breeding gain which this system permits has been widely recognized. Thus, the Japanese paper speaks of relying on foreign thermal reactors for the early stages of the programme, while concentrating Japan's own research and development effort on the design of breeder reactors to be available commercially about 1970.

Reviewing typical national programmes, it is interesting first to compare the countries in which the need for nuclear power is most urgent. The plan to provide 5,000–6,000 MW. of installed capacity in the United Kingdom by 1966 is well known. In Italy, five organizations are each planning to build a station, the combined electrical output being 900 MW., but no precise time-scale is set. The Japanese paper gives detailed economic analyses, both of the cost of stations and the relation of expenditure to the estimated national income. Besides setting targets of 600 MW. by 1965 and 7,000 MW. by 1975, it forecasts that the proportion of equipment manufactured in Japan would rise from 60 per cent in the period 1962–65 to 93 per cent after 1967. The Netherlands, with a present import of about half its fuel supplies and an increasing demand, proposes to have 3,000 MW. of nuclear power station capacity installed by 1975, sufficient to supply 50 per cent of the electrical output. The first step towards this aim is a 100-MW. station in operation by 1962.

France normally imports about 40 per cent of the energy it consumes, the security of which must be dependent on adequate trade balances and also on satisfactory political conditions. The discovery of uranium ores in metropolitan France led to a power programme similar to, but smaller than, the British, in which the most economic system was sought. The early stations, which employ natural uranium and are moderated by graphite and cooled by carbon dioxide, have been designed as prototypes, each differing somewhat from the next (both horizontal and vertical fuel channels have been tried and pressure vessels have been constructed both of prestressed concrete and of steel). The present programme is for two civil power stations to be completed by the middle of 1961 totalling 233 MW. This series of reactors is then to be continued by putting into operation every eighteen months an additional plant, more powerful and more efficient than the preceding one.

Western Germany, with no urgent need to supplement conventional sources of power and a tradition of scientific and industrial ability, plans to investigate a range of reactor systems, including some of its own design, and expects by 1965 to have in operation four or five 'demonstration' stations each producing about 100 MW. of electricity. These stations will be

built for utility companies and not for the Government, though it is proposed that one-third of the capital shall come from public funds. With several research reactors from the United States and the United Kingdom, and bilateral agreements with Canada and the United States for the supply of uranium concentrates and enriched uranium respectively, Western Germany is making great efforts to overcome the handicap of a late start in the field of atomic energy.

At the other end of the scale, both the U.S.S.R. and Poland state that their fossil fuel deposits are adequate and they have no present need for nuclear power, though the Soviet Union makes the exception that it would be useful in certain remote areas. Although they had not reported any new stations since the one they described in 1955, the Russians announced during the Conference the construction of a station in Siberia with an ultimate output of 600 MW.(e). The information given suggests that it is a dual-purpose station with electricity as a by-product. They also showed in the scientific exhibition at the Conference a design for a 150-MW. station using gas-cooled, heavy water-moderated reactors. The Polish programme is based on the conclusion that nuclear stations will be more economic than conventional stations within twenty years, and it is therefore planned to install 800 MW. capacity between 1965 and 1970, with a further 1,000 MW. capacity added in the following five years. It is hoped that the reactors in these later stations may be of the fast breeder type.

An interesting variation is provided by Sweden, where the hydro-electric capacity is adequate to meet demands for the next fifteen years, but where there is virtually no domestic conventional fuel. This lack of coal and oil, combined with the long winter, has caused the Swedish programme to be directed towards the use of nuclear energy for district heating, with the production of electricity by back-pressure turbines as a by-product. By 1965 it is intended to build six reactors to supply heat and only one 100-MW. electrical power station. As the available hydro resources are fully utilized, an expansion in the nuclear power station programme is envisaged, providing a total installed capacity of about 2,000 MW. by 1975.

Other countries, including India, Spain and Roumania, presented papers forecasting the construction of nuclear power stations within the next few years.

The range of experience in operating power-producing reactors is at present both limited and unrepresentative, being based largely on units planned primarily for military purposes or on small-scale demonstration reactors. The 80 per cent availability reported for the Calder Hall reactors is gratifying proof of reliability, but the extremely low incidence of defective fuel elements achieved is not conclusive evidence of performance of the power station because of the relatively low level of irradiation imposed by the military requirements. In the same way, the remarkable performance of the submarine reactors cannot be taken as a reliable guide to the behaviour of a similar type of reactor designed to standards which permit the economic generation of electricity. Thus, both the running costs of the present type of power reactors and their span of useful life, which have an important bearing on the assessment of their economics, can only be finally established when a significant proportion of their estimated life of fifteen or twenty years has been achieved.

Nevertheless, there was at the Conference a high degree of confidence in all the countries concerned that these factors would prove to be favourable to the economic generation of power.

UTILIZATION OF INORGANIC NITROGEN SOURCES BY PLANTS

ONE session of Section K (Botany) of the British Association meeting at Glasgow, held on August 29, was devoted to a consideration of the problems involved in the utilization of inorganic nitrogen sources for amino-acid and protein synthesis by plants. Emphasis was primarily upon the nitrogen fixation process, although recent progress in the use of combined inorganic nitrogen sources was also reviewed.

Dr. G. Bond (Glasgow) stressed that nitrogen fixation constitutes one of the few fundamental processes of the living world and serves to maintain the supply of combined nitrogen in soils and oceans. Without fixation the opposing actions of the denitrifying bacteria would probably reduce the nitrogen status of natural soils to levels incapable of supporting most forms of plant life.

What, then, are the organisms capable of effecting nitrogen fixation ? During a critical review of recent evidence gained largely by the use of the heavy isotope of nitrogen (nitrogen-15), Dr. Bond concluded that no faculty for fixation resides in members of the Angiosperms, Gymnosperms, Pteridophytes or Bryophytes except where there is symbiosis with certain lower organisms. Some blue-green algae (*Nostoc*, *Anabaena* and *Cylindrospermum*) have long been recognized as efficient nitrogen fixers. Certain other members of the Cyanophyceae are incapable of fixing nitrogen, as are all green algae so far tested. The position of the fungi is less certain, and much confirmatory work would seem desirable. While the nitrogen nutrition of most fungi would undoubtedly seem to be based on combined nitrogen, there is preliminary evidence that certain fungi (soil yeasts assigned to the genera *Saccharomyces* and *Rhodotorula*) have some capacity for fixing nitrogen.

The main free-living nitrogen-fixing organisms are without doubt to be found among the bacteria. The genera *Azotobacter* and *Clostridium* include species with an almost world-wide distribution that can effect nitrogen fixation. However, it remains uncertain whether they are sufficiently abundant in soils to permit of extensive nitrogen fixation. Other species for which there is evidence of ability to fix nitrogen include *Azotomonas*, *Aerobacter*, *Desulphovibrio*, *Methanobacterium* and *Pseudomonas* ; positive results have also been obtained for photosynthetic bacteria, including *Rhodospirillum*, *Chromatium* and *Chlorobium*, and so these species match the Cyanophyceae in metabolic powers.

In many plant habitats symbiotic nitrogen fixation is probably much more important than any of the

above types of asymbiosis. Nitrogen fixation by legumes, the root nodules of which contain *Rhizobium* as the endophyte, has been a basic agricultural practice and is still of great importance in certain less industrialized countries. In areas of natural vegetation other Angiosperms capable of forming root nodules may be more important than the legumes. Eight genera so far seem important: these are *Hippophae*, *Elaeagnus*, *Shepherdia*, *Myrica*, *Casuarina*, *Coriaria*, *Alnus* and *Ceanthus*. Undoubtedly the fixation is due to the presence of symbiotic micro-organisms within the nodules, but the nature of these remains uncertain. The importance of certain of these species in the maintenance of nitrogen fertility may well have been greater in earlier times. Alder and bog-myrtle were very prevalent in Britain in pre-agricultural times and they may then have been the principal nitrogen fixers. Recent direct evidence indicates that in Alaska alder figures regularly in plant succession on recently de-glaciated lands and has served to increase soil nitrogen markedly. Sea-buckthorn (*Hippophae*) was another plant that rapidly established itself after the ice-age in Scandinavia and possibly in Britain; this may have been due in part to its possession of nitrogen-fixing nodules.

A number of Gymnosperms, particularly members of the family Podocarpaceae, bear large numbers of nodular structures on their roots. Although reports exist that nodulated plants of *Podocarpus* can continue to grow actively without a combined nitrogen supply, studies with nitrogen-15 have failed to confirm the ability of the plant to fix nitrogen. Various members of the Cycadaceae also produce nodulated roots resembling those of alder. These nodules appear to contain an alga identified as a *Nostoc* or *Anabaena* species, and when detached nodules of *Ceratozamia* and *Encephalartos* were exposed to elementary nitrogen-15, clear evidence of strong nitrogen fixation was obtained. The earlier dominance shown by the cycads in many parts of the world may have been associated with their ability to utilize elementary nitrogen at a time when the combined nitrogen status of most soils was comparatively low.

Several other intriguing possibilities for symbiotic nitrogen fixation exist. The nitrogen-fixing bacterium, *Beijerinckia*, has been isolated from the leaf surfaces of several Indonesian plants and may make symbiotic nitrogen fixation possible. The 'leaf-nodules' (cavities in the leaf surfaces containing dense growth of bacteria) of *Pavetta*, *Psychotria* and *Chromelia* have been assigned nitrogen-fixing ability. Cavities containing nitrogen-fixing blue-green algae are also known; thus, the water-fern *Azolla* is associated with *Anabaena*, while certain liverworts, including *Blasia*, accommodate *Nostoc*. Finally, the lichens seem to provide other examples of symbiotic nitrogen fixation. About 8 per cent of all lichen species contain an algal symbiont of the blue-green type, and recent experiments using nitrogen-15 have provided definite evidence of nitrogen fixation in three such species (*Peltigera*, *Collema* and *Leptogium*). No ability to fix nitrogen resided in lichens containing a green-algal symbiont.

The other papers of the session were concerned with the physiology and biochemistry of nitrogen utilization. Irrespective of whether inorganic nitrogen-15 is supplied as elementary nitrogen, nitrate or ammonia nitrogen, the sequence in which the nitrogen-15 atoms enter organic nitrogen compounds is closely similar for most plants. This suggests that all forms of inorganic nitrogen are converted to a common intermediate before being incorporated into organic compounds. The bulk of the present evidence indicates that this intermediate nitrogen compound is ammonia. Earlier claims that hydroxylamine occupied this key position in the nitrogen fixation process seem to have been discounted.

Dr. G. Leaf (Glasgow) reviewed the older evidence showing that during the fixation of elementary nitrogen-15 by *Azotobacter*, *Clostridium* and soya-bean nodules, the nitrogen-15 probably first entered ammonia and then glutamic acid and the amides, asparagine and glutamine. Similar studies have been carried out in the Glasgow laboratories using nodules from the roots of alder. The pattern of distribution of nitrogen-15 atoms between various nitrogenous compounds differed significantly from that obtained with the legumes. It is noteworthy that citrulline, an amino-acid otherwise rarely encountered, is a major component of these root nodules, and was labelled with nitrogen-15 nearly as heavily as glutamate. Indeed, the labelling of the carbamyl-nitrogen atom (citrulline contains three nitrogen atoms) was substantially heavier than that of the α-amino-nitrogen of the glutamate. This quantitative difference does not, of course, mean that the primary reactions of nitrogen fixation are different in alder, since an established biochemical pathway shows that the carbamyl-nitrogen of citrulline is derived from ammonia via carbamyl phosphate. It is these early reactions and intermediates of the nitrogen fixation process that are still shrouded with mystery. A great deal of speculation exists concerning their nature and many experimental studies have been made to test the validity of the theories propounded, but as yet no unequivocable conclusions have been reached.

A synthesis of various schemes previously published was presented by Dr. Leaf (Fig. 1).

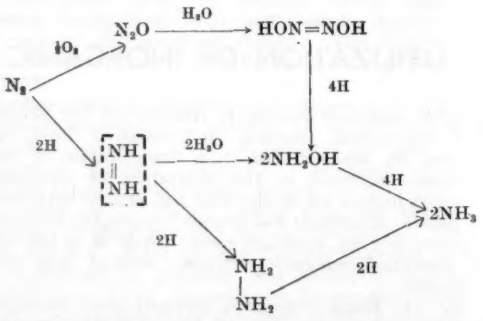


Fig. 1

Uncertainty is encountered in the very first step which can be represented as involving either an oxidation or reduction. Crucial evidence in favour of either mechanism is still awaited. The problem of the intermediates has been approached by testing whether nitrogen-fixing organisms can utilize them, often after they have been labelled with nitrogen-15. The first intermediate of the reduction pathway postulated is not known to exist in a free form and so cannot be detected. Some support is gained for nitrous oxide as an intermediate since it acts as a competitive inhibitor of nitrogen fixation; but since it can only be utilized by *Azotobacter* at a fraction of the rate of elementary nitrogen, more conclusive

evidence would seem desirable. Difficulties arise when later intermediates are tested, for they are often both unstable and toxic to the organism. However, evidence is accumulating to support the intermediary role of hydroxylamine. Hydrazine can also be utilized by *Azotobacter* but, in striking contrast to the assimilatory pathway recognized for elementary nitrogen fixation, the hydrazine-nitrogen was apparently not assimilated into glutamic acid. It is then conceivable that hydrazine is assimilated by processes having no direct connexion with those involved in nitrogen fixation. Thus, it seems probable that the precise details of the chemical pathway of nitrogen fixation will remain unsolved until appropriate conditions are found that enable active fixation to occur in cell-free extracts.

Dr. L. Fowden (London) discussed the assimilatory processes involved in the use of combined inorganic nitrogen sources by plants. The reduction pathway by which nitrate-nitrogen is converted to ammonia seems at last to have crystallized in the following form :

$$HNO_3 \rightarrow HNO_2 \rightarrow HON{=}NOH \rightarrow H_2NOH \rightarrow NH_3$$

Definite evidence is now available from Dr. Nicholas's laboratories demonstrating the existence in fungi (*Neurospora* and *Aspergillus*) of separate enzymes responsible for the catalysis of each reductive step. Naturally, one wishes to see these or similar enzymes established for higher plants. The enzymes, in showing a dependence for various metals, flavin-adeninedinucleotide and reduced phosphopyridine-nucleotides, provide a nice illustration of the importance of trace elements and vitamins in an integrated reaction sequence characteristic of plants.

In higher plants the final reduction product, ammonia, enters organic compounds by being channelled primarily into glutamic acid by reductive amination of α-ketoglutarate or into the amides glutamine and asparagine. Although suggestions are periodically made that alanine and aspartic acid may arise by similar reductive aminations of pyruvate and oxaloacetate, respectively, satisfactory confirmation of these reactions is still required for higher plant systems. Certain mutants of micro-organisms lack glutamic dehydrogenase, and here reductive amination of pyruvate seems to have been established as a major alternative pathway for ammonia assimilation.

Reactions involved in the formation of citrulline and arginine provide secondary pathways for the direct utilization of ammonia. The majority of the other amino-acids required for protein synthesis must gain their nitrogen in a less direct manner. Transamination normally provides the present-day panacea for the problems of their biosynthesis. For this to be effective, it is necessary that all the corresponding keto-acids exist in plants. However, at present only a fraction of the twenty or so acids required have been detected ; it remains possible that the others exist at concentrations that are below the present limits of detection but sufficiently high to enable transamination reactions to provide amino-acids at a rate commensurate with protein synthesis.

In some ways the study of protein synthesis in higher plants is more difficult than in micro-organisms and animal tissues. Plant cells tend to store greater quantities of free amino-acids than the other types of tissue and at present it is impossible to gain a reliable insight into the distribution of particular amino-acids within the different phases of a cell. It would seem probable that in mature cells large proportions of certain amino-acids are deposited in the vacuoles and play no immediate part in protein synthesis. This conclusion receives support in that the relative concentrations of various acids in the free amino-acid pool as measured by extraction bear no similarity to the amounts in which they are required in proteins. Preliminary evidence does, however, suggest that amino-acid activating enzymes similar to those present in animal and microbial systems exist in higher plants and may catalyse the first step in the synthesis of proteins by the proposed and now familiar ribonucleic acid - template pathway.

A brief discussion followed the three formal papers. The participation of more complex organic intermediates in the nitrogen fixation process was considered and dismissed as unlikely. Various alternative concepts of the template mechanism for protein synthesis were also aired. L. FOWDEN

UNIVERSITY DEVELOPMENT IN BRITAIN

By SIR ERIC ASHBY

President and Vice-Chancellor, The Queen's University of Belfast

UNDER the title "University Development, 1952–1957"[1], the University Grants Committee has published a masterly survey of the British universities over the past quinquennium. It is a book of ninety-two pages. Its importance lies not only in the facts which it records but also in its wise and challenging comments.

On the second page is the most significant fact of all : a list of the members of the Committee. Thirteen out of the twenty names are of men and women who are, or have been, university professors or heads of colleges in universities. The controllers of university finance are at one with the controlled ; and upon this simple fact undoubtedly rests the great confidence which universities and the Treasury have in one another. Membership of the Committee must be a strenuous responsibility : over the past quinquennium the Committee met fifty-one times and its sub-committees forty times, and its members spent 107 days visiting 103 institutions. It is a notable record of public service.

The factual part of the book summarizes the growth of universities since 1938–39 : an increase in student numbers from about 50,000 in 1938 to about 90,000 in 1956 ; and in academic staff from about 4,000 to more than 10,000. The average salary paid to academics has about doubled over that period (it was £612 in 1938 and £1,323 in 1956) and the recurrent grant has risen from about £2 million in 1938 to more than £28 million in 1956. In 1938 Parliamentary grants constituted about 35 per cent of the recurrent income of the universities ; in 1956 the per-

centage was more than 70. That such a revolution is being accomplished with so little damage to the integrity of British universities is testimony both to their stability and to the wisdom of successive governments in financing them.

The report records the changes in balance of students entering different faculties over the quinquennium. Arts (which includes law and economics) still has substantially the highest proportion of new entrants (47 per cent in 1953 and 45·6 per cent in 1956). The combined percentage of students entering faculties of science and technology rose from 34·7 in 1953 to 38·7 in 1956. The percentage entering medicine and dentistry fell from 14·6 to 12·6. These figures are reassuring, for they indicate that there is no immediate need to fear that the impact of technology on universities is going to be so violent as to imperil the vitality of more traditional studies. Indeed, there are passages in the report which suggest that the chief danger facing arts faculties may come from within themselves. They may be endangered not by a lack of recruits from the schools nor by competition from other faculties, but (of course, only in some departments in some universities) by an aridity of approach and a poverty of imagination ; a lack of fire in the belly.

It is, perhaps, a philistine approach, but nevertheless one not without its merits, to assess the efficiency of a university system by the percentage of students who emerge from universities with degrees. Compared with American or Australian universities, the record in Britain is good. The report gives some interesting data on this matter, ending with the conclusion that, from a sample of 15,256 students whose records were followed by the Committee, 16·7 per cent left without degrees. This percentage is divided into 11·3 per cent who left through academic failure and 5·4 per cent who left for reasons other than academic failure : a 'wastage' of the same order of magnitude as that reported from the Soviet Union.

One of the features of which Britain can be proud is the way in which State scholarships and local authority awards have brought the universities within reach of almost every young man or woman who is talented and who completes a grammar school education. Three out of every four students at British universities are receiving financial assistance. The proportion varies in different parts of the country : it is highest in Wales, low in Scotland (but this is partly offset by the greater numbers of students per thousand of population in Scotland) and lowest in Northern Ireland (where the sole reason is the parsimony of local authorities). But, as the report points out, the very liberality of local authorities in England causes its problems. Local authorities (quite rightly) allow students to hold awards in any university. This has two consequences : it 'creams off' the best students into a comparatively small number of universities, and so perpetuates inequalities in the intellectual standards of students among universities ; and it increases the pressure on student accommodation, for many students take advantage of this invitation to mobility by attending universities in other parts of the country in preference to universities near their own homes. The Committee sees no remedy to the first of these consequences and it welcomes the second consequence ; though it adds the rider that if more and more students are to leave home to attend universities, the universities for their part must take more trouble than some of them are taking to provide sympathetic supervision, especially during the first year.

The chapter on scientific research in universities has some interesting figures on the amount of research in universities which is sponsored by outside bodies. In the United States a dangerously high percentage of the income of a university may come from this source (one typical private university in the Middle West gets 22 per cent of its recurrent income from outside for sponsored research). The Committee's figures are reassuring : they indicate that only 6·5 per cent of the income of British universities is from this source, and half of that is from the research councils and Government departments, which in general put no restriction on the nature of the research or on its publication. Recently, the Department of Scientific and Industrial Research has taken a much more prominent part than heretofore in giving money both for research projects and for postgraduate awards ; and the Committee very carefully defines its function *vis-à-vis* the Department and the Medical and Agricultural Research Councils over the support of research in universities. The Committee ends by throwing out a challenge which some university departments may be reluctant to accept : a challenge urging universities to regard more favourably than they do at present the possibility of allowing students to work for higher degrees in research establishments off the campus and not administratively under university control.

The chapter on educational problems is convincing evidence (if any were needed) that the Committee is not only a machine for dispensing Parliamentary grants ; it is also a permanent seminar for reflexion on the problems of higher education. Earnest reformers, who would like to see commissions inquiring into university problems in Britain, would do well to ponder this chapter ; for it may well lead them to the conclusion that the sort of inquiries which ought to be made are, in fact, already being made, by a body of people who command the respect of the academic world and the confidence of the Treasury. What does the Committee regard as educational problems ? The pressure (often from professors who have not the intellectual courage to know what to omit from their teaching) to extend courses beyond the common 3-4 years ; the overcrowding of the syllabus (often on the ridiculous assumption that after leaving the university the graduate will never again learn anything new) ; the need to ensure that a graduate has a 'cutting edge' to his mind ; the very controversial question of special entrance requirements and the influence they have on general education ; the problem of teaching arts subjects in an imaginative way with due regard to exactitude and critical alertness ; the unpalatable fact that even a mediocre scientist or technologist acquires a vocational skill, whereas an arts graduate who is not going to use his subject vocationally needs to be more than mediocre—to make his education worth while the arts graduate must acquire from it not just facts (which may suffice for the mediocre scientist or technologist) but genuine mental power.

So one could go on. No virtue, no weakness, no pocket of complacency in British universities escapes the eye of the Committee. To the problems which confront universities in the coming quinquennium the Committee brings wise advice, sympathetic and tactful criticism, and lucid reasoning.

[1] University Development, 1952–1957. (Cmnd. 534.) (H.M.S.O., 1958.)

[See also p. 1210 of this issue]

OBITUARIES

Dr. N. Miller

NICHOLAS MILLER was born in Liverpool on July 4, 1916, and died suddenly at his home in Edinburgh on May 4. Into this short span was crowded a range of experiences and the growth of a reputation that any man might envy. Such was the universality of his talents that, although a classicist at school, he entered the Imperial College of Science and Technology as an entrance scholar in chemistry and had a brilliant undergraduate career culminating in a first-class degree in 1937. After two years research in physical inorganic chemistry he obtained his Ph.D. and a Commonwealth Fund Fellowship. Characteristically, Miller decided on a change not only of scene but also of academic experience, and for two years worked in oil geology and technology in the University of California. In 1941 he joined the Suffield staff of the Canadian Department of National Defence, where his numerous qualities were speedily recognized, and a few years later he was appointed to the National Research Council staff at Montreal participating in the Anglo-Canadian Atomic Energy Project.

In July 1946 Miller was transferred to Chalk River and began those distinguished investigations on the radiation-induced oxidation of ferrous sulphate with which his name will always be especially associated. In 1947 he went as an I.C.I. Fellow to the Department of Natural Philosophy at Edinburgh and in 1949 was made senior lecturer in radiochemistry. During the past ten years he has led a small, but exceptionally effective, group of radiation chemists, and from his pen there came a succession of definitive papers dealing mainly with chemical dosimetry, or more latterly, with the dependence of radical and molecular yields on linear energy transfer. The hallmark of all Miller's work was ingenious, skilfully executed and imaginatively interpreted experimentation described in pellucid prose. His advice was consequently in much demand by national and international bodies, and Miller never shirked the extra work which these duties involved.

Those who knew Miller personally will remember his modesty and integrity, his immense capacity for friendship and his remarkably infectious and tolerant grin which accompanied many a shrewd scientific comment. It is small wonder that some of his English friends have decided to perpetuate his name in annual conferences on radiation chemistry, and that the predominantly North American membership of the sixth annual meeting on Radiation Chemistry of the Gordon Research Conferences should have adopted the following memorial resolution on August 4, 1958:

"Our meeting this year is sadly marked by the untimely death of Dr. Nicholas Miller, late senior lecturer in natural philosophy at the University of Edinburgh.

"Dr. Miller was known affectionately and admired personally and scientifically by the many friends he made in the United States in the course of his repeated travel in this country in the last several years. He participated significantly in current research in radiation chemistry. Through the keenness of his criticism, the precision of his work and the breadth of his experimental and theoretical approach, he made great and lasting contributions to the maturation of the field. The warmth and generosity of his personality and the breadth of his interests outside his work proved a continual inspiration and joy to his colleagues and friends. His death is a loss to radiation chemistry, to science, and to those who had the good fortune to be influenced by him and his work."

F. S. DAINTON

Prof. K. V. Giri

KRAMADHATI VENKATA GIRI was born in 1907 and died suddenly on July 17 at Madras. Since 1950 he had been head of the Department of Biochemistry at the Indian Institute of Science, Bangalore.

Prof. Giri's output of original research extended over a period of more than twenty-five years and covered a wide range of biochemical topics. Phosphatases, especially of leaves, together with free and bound seed enzymes, were early subjects of study. In 1936, interesting observations were made on the amylase system of rice grain during ripening and germination.

Giri made contributions to the analytical chemistry of ascorbic acid and studied its stability under varying environmental conditions. In the early post-war years, vitamin B_1 in ground nut (*Arachis hypogea*) was found to be partly free and partly combined, and a series of studies appeared on the nutritive and vitamin value of this important crop. Giri published a long series of papers dealing with blood and nutrition, in which the results of research carried out in other countries were interpreted and applied to Indian conditions. Many of these papers added important new facts. In the field of the biochemistry of blood and tissues, Giri and his colleagues carried out much work on prothrombin and on nicotinic acid. More recently, radial or 'circular' paper chromatography was applied with considerable success to a variety of separations involving amino-acids, peptides, organic acids and vitamin preparations. For example, free *allo*hydroxy-L-proline was obtained from the leaves of sandal (*Santalum album*); the compound had not previously been obtained from a natural product. Agar electrophoresis was successfully applied to the study of serum of proteins. It was found that in smallpox there was an increase in all the globulin fractions and a decrease in the albumin moiety.

Prof. Giri felt the need to spread biochemical knowledge more widely and made a hobby of devising scientific films. He wanted every medical practitioner in India to have access to clinical biochemical services, and this objective was stressed through his films, writings, radio talks and lectures.

Giri was much liked and his untimely death is a loss to Indian science. He leaves a wife and four children.

R. A. MORTON
H. R. CAMA

Dr. Marie C. Stopes

MARIE CARMICHAEL STOPES, who died on October 2 aged seventy-eight, graduated at University College, London, at the time when Prof. F. W. Oliver, Quain professor of botany, was making notable contributions in the field of palæobotany. As a subject for research, she selected the ovule of the living cycads, and this took her to Prof. Goebel's institute in

Munich. The contemporary work on the Palæozoic ovules by Oliver and his associates had made the elucidation of the structure of cycadean ovules, to which the fossils were superficially compared, a real necessity. Dr. Stopes found evidence in the cycad ovules that the integument is a double structure, and simultaneously with the tentative view expressed by Oliver and Scott, concluded that it is the morphological equivalent of the inner integument and free cupular structure of the *Lagenostoma* ovule: an interpretation fundamental to our understanding of the evolution of integumentary structures.

In 1904 Dr. Stopes was appointed to the staff of the University of Manchester. Among many contributions published during the ten years she spent at Manchester was a treatise jointly with Prof. D. M. S. Watson on the distribution and origin of coal balls. Similar concretions from the Lower Cretaceous of Japan, in which country she spent some time during 1907–9, revealed an extensive flora, and several papers describing this were published, some in collaboration with Japanese botanists.

Her interest in the Cretaceous floras was to become more apparent, for at about this time she was asked to work on the collection of Cretaceous plants housed in the British Museum. The two volumes published as the "Catalogue of the Cretaceous Flora" (1913, 1915) will surely be her best-known scientific work. Preparation for this work took her to many countries. In 1911 she travelled in Canada and the United States, and in between her Cretaceous studies, discovered what were probably the very first coal balls to be found in America, and made collections from the Carboniferous 'fern ledges' of New Brunswick.

Meanwhile, Dr. Stopes had written a small textbook on palæobotany entitled "Ancient Plants" (Blackie, 1910). It was written particularly for elementary students, and was the first English textbook to bring this rapidly advancing subject before students at a non-specialist level.

She returned to University College in 1914 as a Fellow and lecturer in palæobotany. Several papers on Cretaceous plants followed the publication of the British Museum Catalogue, but throughout the succeeding years her interest became more and more centred on coal, quite apart from the plants that went into its making. Spurred, no doubt, by the effects of war, her palæobotanical knowledge was employed in the service of fuel technology. This proved a very fruitful union, and several memoirs, mostly in collaboration with Prof. R. V. Wheeler, are of fundamental importance in this field of work. Their joint "Monograph on the Constitution of Coal" (Department of Scientific and Industrial Research, 1918) set the stage for all subsequent research on this subject. The new terms (clarain, durain, vitrain, etc.) which she introduced later to the petrology of banded bituminous coal found ready acceptance, and her "Classification of Coal Ingredients" (1935) has been almost universally adopted.

Dr. Stopes founded a small museum on Portland Island, and it was one of her ambitions to make it the possessor of the largest collection of Cycadeoidea in England.

D. W. Brett

NEWS and VIEWS

Australian University Grants Committee: Sir Leslie Martin, C.B.E., F.R.S.

In 1956, a committee, appointed by the Prime Minister of Australia, Mr. Menzies, to inquire into such matters as "the role of the university in the Australian community; the extension and coordination of university facilities; technical education at university level; and the financial needs of universities and appropriate means of providing for these needs", was set up. The committee was under the chairmanship of Sir Keith Murray, and its report was published in 1957 (see *Nature*, 181, 300; 1958). The report stated that university development in Australia could no longer be left to individual institutions or confined within the boundaries of one State, and the establishment of a permanent Australian University Grants Committee was strongly urged. This has been accepted by the Australian Federal Government, and Sir Leslie Martin has been appointed the first chairman. Sir Leslie is professor of physics in the University of Melbourne, and, since 1948, has been defence scientific adviser and chairman of the Defence Research and Development Policy Committee. He is an Australian by birth, and was educated at Melbourne High School, the University of Melbourne and Trinity College, Cambridge.

Industrial Biochemistry at Manchester: Prof. T. K. Walker

The retirement of Prof. Thomas Kennedy Walker from the chair of biochemistry in the Faculty of Technology at the Manchester College of Science and Technology brings to a close a long and active career in the service of the College. Although Prof. Walker's earliest publications concerned more particularly the field of organic chemistry, his attention soon turned towards chemical changes effected by various bacteria and moulds with particular reference to those of interest to the brewing and fermentation industries. This is reflected in the prominent position occupied by such topics in a series of almost two hundred publications which appeared in conjunction with various collaborators over a period from the late 1920's to the present day. The importance of his work in this field received recognition in 1956 with his elevation from the position of reader in fermentation processes to professorial status. More recently, the work of his school has received further stimulus from the fact that with the growing recognition of the need for further technological education the Manchester College is being expanded to provide, among other improved facilities, an enlarged school of biochemistry with excellent facilities both for research and teaching. Quite apart from his valuable material services to science, Prof. Walker will take with him into retirement the exceptionally high personal regard of a large number of former students and colleagues.

Prof. A. A. Eddy

The Councils of the University of Manchester and the Manchester College of Science and Technology have approved the appointment of Dr. Alan A. Eddy

to the chair of biochemistry in the Faculty of Technology from January 1, 1959, in succession to Prof. T. K. Walker. Dr. Eddy, who will be thirty-two on taking up his appointment, was educated at Devonport High School and entered the University of Oxford with an open scholarship in natural science in 1945. He graduated in chemistry with first-class honours and later undertook research under Sir Cyril Hinshelwood into the metabolism of alkali metal ions by bacteria, which formed the subject of his thesis for the D.Phil. degree. Having been awarded an Imperial Chemical Industries Research Fellowship, he afterwards participated in the well-known work of Sir Cyril's school on the adaptive behaviour of micro-organisms. In 1954 Dr. Eddy joined the staff of the Brewing Industry Research Foundation, later assuming responsibility for the department of microbiology in which, under the successive directorship of Sir Ian Heilbron and Dr. A. H. Cook, he was able to develop his interest in the chemical aspects of yeast structure. In this connexion his work on the wall of the yeast cell may be specially mentioned as throwing new light on various important aspects of the behaviour of yeast during industrial fermentations, while his development of methods for isolating nuclear bodies from yeast represents an outstanding achievement in a more academic field. Dr. Eddy is the author or joint author of a large number of original memoirs and, in keeping with his future responsibilities, he has been closely associated with various projects for developing the application of continuous methods of fermentation, a subject of growing importance in the industrial field.

Frederick Soddy Memorial Plaque

On October 25, Eastbourne was visited by members of the Soddy Memorial Trust, whose chairman, Sir Gerald Campbell, invited the mayor, Councillor J. W. G. Howlett, to unveil a memorial plaque on the house in Bolton Road where Frederick Soddy was born and passed his early austere and motherless days. At a previous commemoration luncheon, Councillor Howlett expressed the pride of the town in its famous son, who had predicted the wide-scale use of energy from the atomic nucleus. Following a well-attended film show arranged by the Institute of Atomic Information, the Trustees visited Eastbourne College, where Dr. F. M. Brewer was invited to unveil a memorial plaque in the new Soddy Senior Chemistry Laboratory, a notable extension of the laboratories where Soddy began his chemical studies; it has recently been provided by the Industrial Fund for the Advancement of Scientific Education in Schools.

The Second Soddy Memorial Lecture was given by Dr. F. M. Brewer, of the Inorganic Chemistry Laboratory, Oxford, where he became a pupil of Soddy in the early 1920's. He recalled many personal facets of the formulator of the theory of disintegration and of the displacement law, which culminated in the establishment of isotopy and radiochemistry. Soddy always exhibited a great natural charm and generosity of thought, he said, especially towards his students and those with whom he collaborated, which persisted throughout his life and was augmented by the outstanding sociability of his wife. Soddy made little immediate impression on the authorities at Oxford, however, and his extensive work during the First World War at Aberdeen was cut short. Thereafter he became a passionate advocate of the widest use of science for furthering social progress. The local and national neglect of applied science prompted him to investigate political and monetary systems, which provoked academic criticism. Soddy was widely cultured, a mountaineer, an explorer of knowledge in other fields, and a man of the highest integrity, particularly when attributing priority to scientific discoveries and their interpretation. He remains a great man and a fundamental pioneer towards the utilization of nuclear energy. His impact on science is fully documented in "The Life Story of Frederick Soddy", by Muriel Howorth, the first copy of which was presented to the Eastbourne College Library by the author.

Windscale Pile No. 2

The Atomic Energy Authority has decided not to restart Windscale Pile No. 2. The cost of the measures which Sir Alexander Fleck's Technical Evaluation Committee recommended should be taken before restarting Pile No. 2 would be in the region of £500,000. If this cost were incurred and the pile restarted, the plutonium produced over the estimated remaining life of the pile would be extremely expensive. Plutonium is now being produced in substantial quantities from the Calder Hall reactors, and the Chapelcross reactors will come into operation during the next year. The fuel will now be discharged and reprocessed for other uses; equipment will as far as possible be removed, and certain of the buildings will be useful for experimental purposes, saving expenditure which would otherwise have been necessary. This decision will not give rise to any redundancy. The programme of work of the chemical processing plant at Windscale is increasing, and the workers employed on the two piles will be re-allocated to other work.

Use of Radioactive Substances and X-rays in Schools

The Minister of Education, in Administrative Memorandum No. 577 (October 1958) to local education authorities and secondary schools, has announced that, although such work is normally more appropriate to university courses, a small number of schools are introducing demonstrations and experiments involving radioactive substances and sources of X-rays. In view of the possible risks to health of those engaged in such work, and after consultation with the Radioactive Substances Advisory Committee and the Atomic Energy Authority, it has been decided that special authorization will have to be obtained by any school wishing to conduct such work. The Minister will grant authorization, which will be reviewed annually, only where he is satisfied that the necessary safety precautions are maintained. The Minister also requires that he be notified by all schools employing X-ray and other apparatus operating at voltages greater than 5 kV., so that the appropriate protective barriers may be specified, without which such apparatus may not be used. Notes for the guidance of schools in the use of radioactive materials and of equipment producing X-rays have also been issued.

Wellcome Trust: Grants for Research

The Wellcome Trustees have announced that among the grants made during the half-year ended August 31 were the following: up to £60,000 to the Postgraduate Medical School of London for the cost of including a Wellcome Research Library in the proposed new buildings; up to £37,000 to the Institute

of Psychiatry, Maudsley Hospital, to provide laboratories in which integrated research by the Departments of Neuro-endocrinology and Neuropathology may be carried out; up to £36,500 to the Department of Anatomy, University of Birmingham, to extend the research laboratories and animal accommodation; up to £25,000 to the Department of Zoology, University of Edinburgh, to provide additional research accommodation; £50,000 to the University of Western Australia to endow a Wellcome Research Department of Pharmacology; up to £17,000 to the Institute of Medical Research, North Shore Hospital of Sydney, Australia, to build and equip laboratories for experimental medicine and surgery. In addition, three electron microscopes are being purchased at a cost of approximately £34,000 for indefinite loan to the following institutions: the Biophysics Research Unit of the Medical Research Council, King's College, London (Prof. J. T. Randall); Sir William Dunn School of Pathology, University of Oxford (Sir Howard Florey); London School of Hygiene and Tropical Medicine.

Australian Society of Plant Physiology

At the University of Adelaide on August 19, plant physiologists from all the States of Australia attended a meeting convened by Dr. R. N. Robertson, head of the Plant Physiology Unit of the Commonwealth Scientific and Industrial Research Organization in Sydney, to consider the formation of an Australian Society of Plant Physiologists. The sixty scientists present decided unanimously to constitute such a society, with the aim of advancing the science of plant physiology. A constitution was adopted requiring the newly formed society to hold at least one annual meeting which will include a session for the presentation of scientific papers. At the inaugural meeting the following officers were elected. *President*, Prof. J. G. Wood (Department of Botany, University of Adelaide), *Secretary*, Dr. L. H. May (Waite Agricultural Research Institute, Adelaide), *Treasurer*, Dr. L. G. Paleg (Waite Agricultural Research Institute, Adelaide), *Committee Members*, Dr. N. P. Kefford (A.C.T.), Mr. L. A. Thomas (Queensland), Dr. J. F. Turner (New South Wales), Dr. P. Brownell (South Australia), Dr. D. M. Paton (Tasmania), Dr. D. J. Carr (Victoria), Prof. B. J. Grieve (Western Australia).

Some scientific papers were also presented at the inaugural meeting, and these indicated the wide range of interests of Australian physiologists. Nitrogen metabolism and the mechanism of ion uptake by plant cells, which have long been under study in Adelaide and Sydney respectively, were discussed in the light of recent work. Growth regulators formed the basis of several papers, ranging from synthesis in a symbiotic association, effects in photoperiodic and vernalization responses, to their effects in root contraction. Translocation, plant cell structure, sodium nutrition and the dynamics of growth in seedlings were among other topics presented. Abstracts of all the papers will be published in the *Australian Journal of Science*. At the next meeting, to be held in Canberra, it is planned to include agronomic and horticultural topics of interest to plant physiologists.

Forests Products Research Laboratory

The Council for Scientific and Industrial Research has set up the following Steering Committee to look after the research programme of the Forest Products Research Laboratory, in place of the Forest Products Research Board: Dr. B. K. Blount, deputy secretary of the Department of Scientific and Industrial Research (*chairman*); H. Douglass, a member of the Research Council and general secretary of the Iron and Steel Trades Confederation; L. J. Cardew Wood, managing director of Bell's Asbestos and Engineering, Ltd., and director of Bell's Asbestos and Engineering (Holdings), Ltd.; the director of the Forest Products Research Laboratory (Dr. F. Y. Henderson); H. Wooldridge, head of the Stations Division, Department of Scientific and Industrial Research. Its terms of reference are: (1) to be responsible to the Research Council for the selection of projects and for the allocation of effort to them, within the financial resources allocated to the Laboratory and such other limits as may from time to time be imposed by the Council; (2) to report annually to Council, submitting a research programme for the following year. The Committee, which will be a full committee of the Research Council, is empowered to set up specialist sub-committees to provide technical advice on particular fields of work and especially on research in the field of pulping.

Future of Timber Research in the United Kingdom

Following recent announcements of its policy on forest products research, the Council for Scientific and Industrial Research recommends that the ultimate objective should be to concentrate all timber research at Princes Risborough, the site of the present Forest Products Research Laboratory. This would avoid the danger of overlap and unnecessary expense that might arise if separate laboratories were developed in different localities to serve the sectional interests of the timber trade and would ensure that the most effective use is made of the qualified staff and material resources available in the various organizations concerned with timber research. Discussions are being held with the Timber Development Association and the Forestry Commission to determine the type of organization which would most effectively co-ordinate the research requirements of their interests. As an initial step to the achievement of this objective, the Department of Scientific and Industrial Research is discussing with the Timber Development Association the possibility that part of the Princes Risborough site might be made available to the Research and Development Committee of the Association for immediate development, without prejudice to work at present in hand at its existing laboratories. Discussions will shortly be held with other trade organizations interested in timber research.

Royal Observatory, Cape of Good Hope

In his report for 1957 H.M. Astronomer at the Cape of Good Hope records continued progress in many branches of astronomy (pp. 9. Cape of Good Hope: The Observatory, 1958). On the administrative side the increase in the number of visiting astronomers from outside South Africa and the growing co-operation between the Cape and Radcliffe Observatories are both valuable developments. The routine work of the Cape Observatory is undoubtedly the most important, and in some fields it is the only work of its kind being done in the southern hemisphere. The meridian circle programme of observations of about seven thousand stars is approaching completion, and a new working list of faint stars for use as standards in photographic plate

reductions is being prepared. The preparation for press of the "Cape Photographic Catalogue", zones −56° to −60° and −60° to −64°, has been completed; astrometric work on the zone −64° to −68° was completed in 1955 and the photometric work for this zone is almost finished. Position measurement for 4,400 stars in the −68° to −72° zone was completed, and proper motion and magnitude measurements are in progrsss. Preliminary work has been undertaken in all the remaining zones to the south celestial pole, involving 9,500 stars in all. A large programme of photoelectric photometry is under way with several telescopes. Magnitudes are being determined for many standard stars, stars on radial velocity programmes, parallax stars, stars in selected areas, comparison stars for cepheid variables, southern stars in the "Yale Bright Star Catalogue", and for other interesting objects. More than 7,000 observations were made during the year; more than 50,000 images were measured in a programme of photographic photometry, and other routine observations of the Sun, variable stars and occultations were continued. The radial velocity work at the Radcliffe Observatory was concerned with nearby stars, stars in the southern galactic cap and standard stars. Some miscellaneous observations were made on extragalactic nebulae, and the first section of the "Cape Photographic Atlas of Southern Galaxies" was published during the year.

Society of Protozoologists

THE following elections have recently been made by the Society of Protozoologists: *President*, Dr. Elery R. Becker (Arizona State College); *Vice-President*, Dr. Norman D. Levine (University of Illinois); *Secretary*, Dr. John O. Corliss (University of Illinois); *Treasurer* (retained), Dr. Daniel M. Lilly (St. John's University, Jamaica, New York); *Editor* and *Acting Editor* of the *Journal of Protozoology* (both retained), Dr. William Trager (Rockefeller Institute) and Dr. Seymour H. Hutner (Haskins Laboratories, New York City); *Honorary Member*, Dr. Emmanuel Fauré-Fremiet (Collège de France, Paris). The Society will hold its next annual meeting with the American Institute of Biological Sciences in August 1959 at Pennsylvania State University.

The Night Sky in November

NEW moon occurs on Nov. 11d. 06h. 34m. U.T., and full moon on Nov. 26d. 10h. 16m. The following conjunctions with the Moon take place: Nov. 13d. 16h., Saturn 4° S.; Nov. 25d. 07h., Mars 3° N. In addition to these conjunctions with the Moon, Mercury is in conjunction with Antares on Nov. 11d. 12h., Mercury being 2·1° N. Mercury and Venus are too close to the Sun for observation. Mars rises at 17h. 25m., 16h. 05m. and 14h. 45m. at the beginning, middle and end of the month, respectively, setting on these dates at 8h. 55m., 7h. 30m. and 6h. 10m.; conditions for observation are very favourable. Mars reaches opposition on November 16, when its distance is 46 million miles and its stellar magnitude −2·0. Mars continues to retrograde, moving from Taurus into Aries on November 22. Jupiter is too close to the Sun for observation. Saturn may be visible low in the south-west after sunset, but conditions for observation are not favourable. Occultations brighter than magnitude 6 are as follows, observations being made at Greenwich: Nov. 2d. 02h. 17·1m., 26 Gem. (*R*); Nov. 3d. 01h. 43·4m., 68 Gem. (*R*); Nov. 27d. 20h. 58·6m., 115 Tau. (*R*); Nov. 29d. 23h. 05·0m., λ Gem. (*D*); Nov. 30d. 00h. 17·9m., λ Gem. (*R*). *R* and *D* refer to reappearance and disappearance, respectively. The Taurid meteors are active during the first fortnight of the month, conditions being favourable; the radiant is near R.A. 3h. 36m., Dec. +14°. The Leonid meteors are active on November 15–17, conditions being favourable; the radiant is near R.A. 10h. 08m., Dec. +22°.

Announcements

MR. W. A. DUTTON, assistant director of the Hosiery and Allied Trades' Research Association, Nottingham, and Mr. H. Marsden, Universal Winding Co., Ltd., Manchester, have been awarded the Institute Medal of the Textile Institute. This Medal was inaugurated in 1921 as an award by which recognition could be given for distinguished service to the textile industry in general and to the Institute in particular.

TRINITY COLLEGE, Cambridge, offers entrance exhibitions to male students of universities in the British Commonwealth to enable them to read for the degree of B.A., or LL.B., or for a diploma. Further information can be obtained from the Senior Tutor, Trinity College, Cambridge.

A CONFERENCE on Electronic Digital Computers and their Industrial Applications will be held in the Department of Mathematics, Birmingham College of Technology, on December 10, when members of IBM, Ltd., will speak on the following topics: data processing machines available; commercial applications on IBM 650; technical applications including linear programming; organization and preparation for electronic data processing. During the course of the day, three 16-mm. sound films to illustrate IBM machines will be shown. The conference fee is £2, and further details and application forms can be had from the Registrar, College of Technology, Gosta Green, Birmingham 4.

THE Faraday Society Bourke Lectures will be delivered by Prof. R. H. Stokes (University of New England, Australia). The lectures will be: "Tracer Diffusion and Self-diffusion in Concentrated Electrolytes", in the Main Lecture Theatre, Chemistry Department, King's College, Newcastle on Tyne, on November 14, at 5.30 p.m. (chairman: Prof. W. F. K. Wynne-Jones); and "The Relation between Ion Mobility and Viscosity in Aqueous Solutions", in the Chemistry Department, University of Glasgow, on November 18, at 4 p.m. (chairman: Prof. J. Monteath Robertson). The second lecture will also be given at the Edward Davies Chemical Laboratories, University College of Wales, Aberystwyth, on November 20, at 5.15 p.m. (chairman: Prof. C. W. Davies). Further information can be obtained from the Faraday Society, 6 Gray's Inn Square, London, W.C.1.

WE regret that in referring to the appointment of Sir Rudolph Peters as president of the International Council of Scientific Unions (*Nature*, October 11, p. 990), he was wrongly described as lately director of the Agricultural Research Council Institute of Animal Physiology; Sir Rudolph is, in fact, head of the Biochemistry Department of the Institute.

ERRATUM. In the communication entitled "Stability of Evaporated Films", by M. Wyn Roberts, in *Nature* of October 25, p. 1151, par. 4, line 11, for "33×10^{-3} cm.^{3} (S.T.P.)" read "13×10^{-3} cm.^{3} (S.T.P.)".

THE CHEMICAL RESEARCH LABORATORY

REPORT FOR 1957

THE report of the Director of the Chemical Research Laboratory which accompanies the report of the Chemistry Research Board under the title "Chemistry Research, 1957"* is the last which will cover the work of the laboratory under that title, which has now been changed to the National Chemical Laboratory, and the Board itself was being disbanded on September 30 and replaced by a Steering Committee. The new unit at Harwell, which is to co-operate with the Atomic Energy Authority, is studying possible applications of radioisotopes and began work there on October 1, 1957. To permit extension of the programme carried out for the Authority on the extraction of radioactive elements from minerals and ores the Authority is extending the Radiochemical Building and increasing the staff engaged on its behalf. The Authority is also contributing to the intensification of work on chelating and complexing resins. After a critical review during the year of the scheme of providing standard organic compounds, the Board agreed that the charges made for pure standard samples should fully cover the cost of the service, and to economize scientific effort recommended that the preparation of standard materials should be limited to those not available by purchase elsewhere. The Board's report also emphasizes the urgent need to expand work in the inorganic field.

The Director's report notes the increased effort given during the year to the work on the extraction, concentration and purification of elements from ores and low-grade materials, the preparation of ion-exchange membranes and the synthesis of complexing resins. Among advances to which he directs particular attention are the construction of three experimental water-circulating systems to study the failure of tubes by corrosion, in water-circulating systems of modern engineering projects, and the development of an experimental technique to give reproducible results. The formation of an absorbed film and the subsequent growth of an oxide film have been followed by radiometric methods. A rapid method has been developed of concentrating the less plentiful constituents of rare-earth mixtures using a partial chromatographic technique, and further studies of zone-refining have shown that with tellurium, indium, tin and antimony the method is not effective in removing all the impurities as required by the electrical industry. The possibility of recovering germanium from ammoniacal liquor has also been examined. Advances are reported with resin-in-pulp and solvent extraction processes for extracting and recovering uranium and thorium from ores, and an ion-exchange phosphorylated cellulose has been developed for recovering thorium from sulphate solutions of monazite as well as a field method, which can be used by relatively unskilled personnel, for determining as little as 0·001 p.p.m. of uranium in river water.

The work of the Organic Group has included a new study of the synthesis and application of certain organo-metallic compounds with special reference to the preparation of materials of high thermal stability, further work on the isomerization of ethyl*cyclo*pentane, the reactions of nascent carbon-14, the rearrangement of dipotassium salicylate and hydrazine polymers and the polymerization of aromatic *iso*cyanates. Progress is reported in the purification and measurements work, including the construction of a high-voltage paper-electrophoresis apparatus and apparatus for the direct measurement of latent heats of vaporization and specific heats of vapours. Encouraging progress is also reported in the preparation of new types of ion-selective membranes, and cation- and anion-selective membranes based on polyvinyl chloride have shown very promising mechanical and electrochemical properties. In fractionating polymers it has been found that ^{14}C-labelled polysarcosine dimethyl amides of molecular weights up to about 12,000 can be separated by paper chromatography using gradient elution, and studies on the random partial sulphonation of cross-linked polystyrene promise to throw light on the fundamental mechanism of ion-exchange.

A comprehensive collection of organisms used in the assay of amino-acids was built up in the National Collection of Industrial Bacteria during the year. Pilot-plant trials of the microbial production of sulphur from sulphate-enriched sewage sludge in progress at the London County Council's Northern Outfall Works at Beckton have confirmed earlier laboratory experiments in that the final sludge 'dewaters' more efficiently than sludge digested from methane. The great reduction in the volume of solids for disposal and consequent saving in transport costs may have an important influence on the economics of the process. Other work by the Microbiology Group has been concerned with the microbial formation of methane and with the bacterial oxidation of aromatic compounds, including benzenesulphonic acid and catechol, and the perfusion apparatus described in the 1956 report has been used to satisfy requests from industry for organisms capable of destroying such recalcitrant chemicals as methylated and unmethylated aryl sulphonates, thiocyanates and β-naphthol.

The Chemical Engineering Group is studying the rate of solution of gases in agitated liquids and has devised an optical device for measuring the interfacial area in gas–liquid dispersions. It has also obtained valuable data on the evaluation of mass transfer coefficients.

About 10 per cent of the time of senior staff is occupied with advisory work, and at the end of the year the total staff, including 58 scientific officers, 86 experimental officers, 34 assistants (scientific) and 2 works technical officers, numbered 239 compared with 223 at the end of 1956. Appendixes list the staff in post on December 31, 1957, as well as the committees of the Board, patents publications during the year, standard samples of organic compounds and organisms deposited in the National Collection of Industrial Bacteria.

* Department of Scientific and Industrial Research. Chemistry Research 1957: The Report of the Chemistry Research Board with the Report of the Director of the Chemical Research Laboratory. Pp. iv+96. (London: H.M. Stationery Office, 1958.) 5s. net.

SALT AND WATER BALANCE IN ANIMAL LIFE

THE mechanisms by which salt and water balance is maintained in the majority of the groups of cœlomate animals are now fairly well understood. Such mechanisms are of importance not only in all freshwater animals, which must always protect themselves against the continual loss of salts and the uptake of water, but also in many marine animals, where it is found that the salt and water content of the body is also carefully regulated. There are, however, many aspects of the functioning of these mechanisms about which very little is known at present. For example, salt balance is generally maintained by the activity of a specialized group of cells which are localized, often in the respiratory membranes, and which are capable of doing work to sustain a gradient of salt concentration across the membrane. Although the gross location of these cells can generally be inferred, we still have very little knowledge of their nature and properties at a cytological level. Histological and cytochemical studies under conditions of varying salt concentration can help in their identification and characterization.

The quantitative measurement of salt and water movements in animals is another important aspect which, as yet, has received little attention. It is only by such measurements that it is possible to discover the factors upon which these movements depend and the extent to which they can be regulated and controlled by the animal itself. The general availability of radioactive and other forms of tracer substances has now made this type of measurement much easier.

Both cytological and quantitative investigations of the mechanisms of salt and water balance in animals were considered in a symposium under the above title which was held as a joint session of Sections *D* and *I* (Zoology and Physiology and Biochemistry) at the British Association meeting at Glasgow on September 1.

Dr. T. Vickers (University of Cambridge) took up once again the question of the cells responsible for salt secretion in the teleost fish. Nearly thirty years ago Homer Smith showed that in the marine teleosts salt and water balance is achieved by the ingestion of large amounts of sea-water, the intestinal absorption of water and salts from this and the subsequent extrarenal excretion of the excess salt. Keys demonstrated that this salt was secreted by the gills, and Keys and Willmer identified large eosinophil cells in the branchial epithelium of the eel and suggested that these cells were responsible for salt secretion. Vickers showed that a similar type of cell appears in the gill lamellæ of the brackish- and fresh-water guppy, *Lebistes reticulatus*, when this is adapted to salt solutions. Under these conditions, small, previously undifferentiated cells are mobilized and they develop into large eosinophil cells the size of which depends on the salt load. They appear to be active cells with many mitochondria but give negative tests for mucus when fully developed. During the normal development of these cells they have small pits at their distal ends which are mucus-positive but which disappear as the cell enlarges. It is interesting that if the animal fails to adapt itself to the new salinity these pits enlarge and become filled with mucus, the cell cytoplasm becomes reduced and the cell now strongly resembles a normal goblet cell. It is clear that in this epithelium transformation from one functional cell type to another can take place. The primary stimulus for the formation of the salt-secreting cell is presumably the increase in salt load, but whether this can directly affect the branchial epithelium or whether the response is induced indirectly is not yet known.

That it is possible to induce a transformation of epithelial cells directly was demonstrated by Dr. H. B. Fell (University of Cambridge). She showed that vitamin A controls the differentiation of certain types of epithelia, the respiratory epithelium being especially susceptible. Thus, for example, the epithelium of the trachea is composed typically of columnar, ciliated and mucous cells. In the absence of vitamin A the ciliated and mucous cells disappear and a squamous epithelium is formed. The direct nature of this effect can be demonstrated by the behaviour of embryonic chick skin in tissue culture. A keratinizing epithelium of the squamous type is formed in a normal culture medium, but if vitamin A is present the keratin process is suppressed and the cells produce a mucous secretion. In some cases ciliated cells may also be produced. If the tissue is returned to the normal medium, after a few days of intensified secretion the basal cells proliferate and the squamous epithelium is regenerated.

Dr. R. Morris (University of Nottingham) showed that cell changes occurred in the respiratory epithelium of the river lamprey, *Petromyzon fluviatilis*, also. He described how the adult lampreys, which are found in the sea and in estuaries, come upstream to spawn and during the process lose their marine osmoregulatory mechanism, replacing it by a fresh-water one. Animals taken from fresh water vary greatly in their behaviour in 33 per cent sea-water. Mature animals lose much water and their blood concentration rises to that of the outside solution. The fresh-run animals are very variable: some behave like mature animals, whereas others are able to maintain both salt and water balance. In the latter case, the mechanism closely resembles that found in the marine teleost and, in fact, the lampreys only differ in their greater water permeability. Just as in the marine teleost the gill epithelium contains salt-secreting cells, but its nature is variable. In many fresh-run animals the secreting cells are regressing and other cells, which are typical of the freshwater animals, are taking their place. The breakdown of the marine mechanism involves not only the disappearance of the secretory cells but also the loss of swallowing capacity as the animal ceases to feed in fresh water and the gut degenerates and there is an increase in permeability of the body surface.

The lampreys probably developed in fresh water and evolved their marine osmoregulatory mechanism quite independently of the teleosts.

The extent to which a knowledge of the mechanism of salt and water balance in the lower vertebrates, such as the cyclostomes and fishes, can assist in deducing the type of habitat in which the vertebrate

group as a whole evolved was discussed by Dr. J. D. Robertson (University of Glasgow). A widely held view, due mainly to Homer Smith and Marshall, is that certain features of these mechanisms, especially the presence of the peculiar glomerular kidney, point to a freshwater origin for the vertebrates. Robertson challenged this view. He showed that the palæontological evidence indicates that the earliest vertebrates, the Ostracoderms and the Acanthodians, lived in the coastal waters of the sea, and their remains, found in the rocks of the Ordovician and Silurian periods, are mostly associated with known marine invertebrates. At the same time, geochemical evidence suggests that the seas in Ordovician times were probably almost as saline as they are to-day. Robertson claimed that the inferences which could be drawn from the physiological features of present-day vertebrates are not opposed to this view. He brought forward the fact that all non-vertebrate chordates are marine and their blood composition, where known, is similar to that of sea water. Although the salt content of the blood of almost all the aquatic vertebrates is much less than that of sea water, the blood of the myxinoid cyclostomes—a very primitive vertebrate group—is not dissimilar, and this may be a primary feature. Finally, he suggested that the glomerular kidney is not necessarily a freshwater adaptation. It may well have existed in marine protovertebrates as it does to-day in the marine elasmobranchs and myxinoids. In these marine animals the glomerular filtration surface may be practically as large as that of the freshwater teleosts.

There seems little to be gained by pursuing this question further until new facts have come to light. On the physiological side, many more studies on the quantitative aspects of renal function in the cyclostomes and fishes would do much to increase our knowledge on this fundamental issue.

A quantitative approach to the problems of salt balance was adopted by Dr. J. Shaw (King's College, Newcastle), who described an analysis of sodium uptake by the freshwater crayfish, *Astacus pallipes*. A steady state is achieved when the normal sodium loss is balanced by an equal uptake of sodium from the environment. The rate of sodium uptake can be conveniently measured by the use of a radioactive sodium isotope. The rate of sodium uptake depends on the external sodium concentration and a maximum rate is approached at concentrations as low as 1 m.mole/l. Above a certain minimum value balance can be achieved at any external concentration, since the uptake-rate is also regulated by the internal sodium concentration. A fall in the blood sodium concentration of 10 per cent or less may increase the rate of uptake by as much as five times. The system is a self-regulating one and results in the maintenance of the blood sodium concentration despite changes in the sodium content of the environment or the presence of other factors which may affect the sodium uptake-rate.

J. Shaw

AQUATIC AND AIR POLLUTION

A SYMPOSIUM on "The Effects of Pollution on Living Material", organized by the Institute of Biology, was held at the rooms of the Royal Geographical Society on September 25 and 26. The general and scientific interest in various aspects of this subject may be gauged from the fact that 380 registrations were received, more than a hundred of which were from industrial organizations, public authorities and similar bodies. The symposium was divided into four sessions, each dealing with one separate aspect of the problem: these were river, estuarine and air pollution, and pollution by radioactive substances.

In the field of air pollution the immediate need is clearly to study its effect on man, but there was evidence at this symposium that other aspects of the problem are not being neglected. Recent studies by Gorham[1] suggest that air pollution is increasing the concentrations of sulphuric acid in atmospheric precipitation in the Lake District and "will presumably hasten deterioration of already heavily leached Lake District soils". Moorland and high tarn communities may well be affected. Changes of this sort brought about by air pollution, though difficult to study except in local areas and special habitats, may be widespread.

Before about 1945 the biologist studying water pollution was primarily interested in the effects of polluting discharges on river and estuarine communities. The change in community structure often gave a useful measure of the severity of the pollution. The mode of action of the pollutants which resulted in such changes was rarely studied and, with the exception of organic pollution associated with low oxygen concentrations, practically no information was gathered on how these selective forces operated at organismic level. This state of affairs is generally true to-day, although a recent advance has been made concerning the chemical factors important in the distribution and deaths of fish in a sewage effluent channel[2].

Since the end of the Second World War, however, there has been an increasing interest in the effects of organisms on the polluted environment. In pollution by radioactive wastes, although there is no evidence of changes in community structure, the activity of organisms in accumulating and afterwards releasing radioisotopes is of the utmost importance in determining permissible discharges. In the field of 'conventional' water pollution similar trends are evident, and it seems likely, from the papers presented, that in the near future it will be possible to make estimates of some of the effects which organisms have on their environment.

The first speaker in the session concerning river pollution, Mr. F. T. K. Pentelow (Ministry of Agriculture, Fisheries and Food), began by admitting the difficulty of defining pollution. It could be said, however, that water is polluted when it becomes unfit for some use to which it could be put in its natural state. Mr. Pentelow directed attention to the many improvements which have occurred in British rivers over the past thirty years at a time of great industrial and urban expansion in spite of a major slump, rearmament, war, and a series of financial crises. Pollution, though now perhaps more widespread, is not nearly so intense as at the beginning of this period. Mr. Pentelow considered that an important factor leading to further improvement has

been the recent formation of river boards and their endowment with statutory powers of control.

Mr. H. B. N. Hynes (University of Liverpool), discussing the biological effects of water pollution, suggested that the effects vary with the age of the phenomenon which the pollution produces. Man-made types of pollution, such as the discharge of poisons, which have no counterparts in natural ecological systems, result in an elimination of species, whereas other types, such as organic and thermal pollution, result in the development of replacement communities adapted to the peculiar ecological conditions. These observations seem to provide a useful basis for an ecological classification of pollutional effects. Dr. Hynes suggested that a controlled introduction of selected organisms tolerant of the somewhat higher temperatures found near discharges of cooling water from power stations might be considered. There are severe objections to such introductions, however, some of which were raised by the delegates present.

Mr. D. F. Westlake (Freshwater Biological Association) described recent studies made to assess some of the effects which plants and invertebrates have on stream conditions. It was shown that these organisms have quite large effects on the oxygen regime of organically polluted streams where high densities of bottom-living invertebrates and often large growths of macrophytic vegetation, especially *Cladophora*, occur. Estimates of these changes in the concentration of dissolved oxygen based on field observations were presented, together with descriptions of experimental studies of the effects of some invertebrates on bottom deposits. Physical effects of plant growth in streams were also discussed.

In the discussion which followed this session, Mr. F. W. Roberts (Luton Sewage Works) described his experiments on the effects of detergents on aquatic plants and reported that when grown in concentrations of 2·5 p.p.m. of anionic detergents, certain plants, such as *Ranunculus* and *Apium*, showed browning and loss of leaves. A non-ionic detergent did not have the same adverse effects. The tolerances of different plant species to detergents reflected their natural distribution in the river system with which he was concerned.

The second session, dealing with estuarine pollution, was introduced by Dr. B. A. Southgate (director, Water Pollution Research Laboratory), who said that British estuaries were generally in a worse condition than the rivers. He attributed this principally to differences in legislation and to past over-assessment of the capacity of estuaries to deal with pollution.

The first two speakers in this session, Mr. A. B. Wheatland and Mr. A. L. H. Gameson (Water Pollution Research Laboratory), discussed some aspects of the carbon, nitrogen and sulphur cycles in the Thames estuary. Mr. Wheatland described his investigations on denitrification and particularly the influence of low concentrations of dissolved oxygen and organic matter on this process. Some of the factors affecting the formation and oxidation of hydrogen sulphide were also described. Mr. Gameson discussed the importance of the biochemical reduction of nitrate and sulphate, and photosynthesis by phytoplankton, in contributing oxygen to the Thames Estuary. The reduction of nitrate produced about 140 tons of oxygen daily, that is, roughly one-quarter of that supplied by absorption from the atmosphere. Sulphate reduction and photosynthesis would seem to play a minor part in the oxygen balance of this estuary.

The final speaker on the first day, Prof. G. E. Newell (Queen Mary College, London), confining his remarks to lower estuaries, described the eutrofication resulting from organic pollution and its enrichment of the fauna and microflora. It was shown how hydrographic characteristics of estuarine basins and condition of the deposits may influence these pollutional effects.

In the discussion which followed, Mr. R. Bassindale (University of Bristol) showed how difficult it was to decide, in any given case, how far pollution was responsible for the reduction in fauna in the middle reaches of an estuary, for fluctuations in salinity in these areas result in a similar faunal reduction, and pollutional effects are generally superimposed on natural salinity effects.

The morning of September 26 was devoted to a series of papers on air pollution. The first speaker, Dr. E. T. Wilkins (Fuel Research Station), described the methods of measurement of deposited matter, sulphur dioxide, and smoke, and commented upon the value of the instruments employed for this purpose—now almost 3,000 in Great Britain—in monitoring suspected sources of excessive pollution and assessing local and national trends. Dr. Wilkins described measurements of toxic constituents of motor-vehicle exhausts, the most harmful of which appears to be carbon monoxide, which reaches concentrations of 50 p.p.m. at breathing height in London streets congested with traffic. The general conclusions of smog studies were outlined and possible synergistic effects of smoke, sulphuric acid and other complexes were discussed. The possibility of neutralizing acid gases in smog by the controlled addition of ammonia is being explored.

Dr. J. K. A. Bleasdale (National Vegetable Research Station) described his studies of the deleterious effects of air pollution on plant growth, these being particularly severe on winter annuals, which grow at a time when air pollution is at its worst. Dr. Bleasdale discussed the toxic effects of pollution on plants and soils and the effect of smoke haze in reducing light available for photosynthesis. The one redeeming feature in this otherwise gloomy picture is the notable freedom from certain fungal infections of plants growing in polluted air.

Dr. P. J. Lawther (Medical Research Council) pointed out that while acute air pollution is manifestly injurious and even fatal to old people and respiratory cripples, less dramatic consequences such as the hastening of the development of chronic bronchitis must not be overlooked. The chronic pollution suffered by town-dwellers is also suspected as an etiological factor in the production of chronic bronchitis and lung cancer, though in the case of the latter disease air pollution can play only a small part in the alarming increase in its prevalence.

Dr. R. Allcroft (Ministry of Agriculture, Fisheries and Food), reading a paper entitled "Fluorosis in Farm Animals", briefly surveyed the areas of occurrence and the industries with which it is associated. In Britain, fluorosis results exclusively from the aerial contamination of herbage by the emission of gases and dusts containing fluorine. The symptoms of fluorosis in cattle and other farm animals were described and shown on film, and the effects of dental changes and lameness, associated with fluorosis, on the quality of stock were discussed. Adverse environmental factors and physiological stresses greatly

influence the severity of the toxic effect and it seems difficult to define damaging pasture concentrations, but a tolerance-level of between 30 and 50 p.p.m. in the food was given.

Mr. R. H. Burns (Atomic Energy Research Establishment), the first speaker in the final session, devoted to pollution by radioactive wastes, outlined the main differences between radioactive and other waste materials, the most important being our inability to destroy the harmful properties of the former by chemical means. Methods of dealing with wastes, disposal and storage, were described. There is an increasing demand for some of the fission products, particularly radiocæsium and radiostrontium, in industry and medicine, and storage of liquid wastes, though expensive, costing £200 per cubic metre, is being used on an increasing scale. Mr. Burns demonstrated by his paper how carefully such factors as the proper siting of effluent pipes and the fixing of permissible levels of discharge are considered in relation to the disposal of radioactive wastes.

Mr. W. L. Templeton (U.K. Atomic Energy Authority) directed attention to the greater hazards that may arise from accumulation of radioactive isotopes from the diluting medium by food-organisms than by direct radiation from the environment. The value of the observational and experimental approaches in the measurement of accumulation factors was outlined and their findings surveyed. Mr. Templeton felt, however, that series of planned experimental discharges, each of several months duration, would be of the greatest value in determining long-term variations and build-up of activity.

Dr. H. J. M. Bowen (Wantage Radiation Laboratory), discussing the uptake of fission products by plants, outlined recent advances in our knowledge of their absorption by various plant structures and their translocation in plant tissues. Dr. Bowen described his work with accumulator plants, that is, plants which concentrate large quantities of specific elements. He found that radiostrontium, the most dangerous fission product, was accumulated particularly by certain species of *Fucus* and *Laminaria*. It would seem that one important gap in our knowledge is that of the relation of rainfall and soil-type to the accumulation of fission products by plants.

In the last paper of the symposium, entitled "Genetic Hazards of Radioactive Pollution", Dr. T. C. Carter (Medical Research Council) suggested that recent work casts doubt on assumptions made by experimental geneticists, based principally on the radiation effects on gene mutation in *Drosophila* sperms. It is on these questionable assumptions that radiation effects are calculated. Dr. Carter stressed the need for an investigation of the relation between rates of mutation and accumulated radiation doses in mammals, particularly in the low-dosage range relevant to man. All present-day assessments of genetic hazards of radiation assume a linear relation. A figure of about £200,000 was quoted as the total outlay necessary for such an investigation. This is surely a small price to pay for such basic and vital information, especially when man's future well-being is at stake.

R. W. Edwards

[1] Gorham, E., *Trans. Roy. Soc.*, B, **241**, 147 (1958).
[2] Allan, I. R. H., Herbert, D. W. M., and Alabaster, J. S., *Fish. Invest.*, **8**, No. 2 (1958).

THE UNIVERSITY GRANTS COMMITTEE

RETURNS FOR 1956–57

THE annual returns from universities and university colleges in receipt of Treasury grants for the academic year 1956–57, published by the University Grants Committee*, records a further increase of 4,672 in the number of full-time students, which now stands at 89,866, compared with 81,705 in 1954–55, and it is expected that the number in 1957–58 will show a greater increase since the number of full-time students in the autumn term of 1957 was 5,899 greater than in the autumn term of 1955. There were 6,115 full-time and 2,016 part-time students from overseas within the British Commonwealth and 3,792 full-time and 1,756 part-time students from foreign countries; for 1955–56 the corresponding figures were 5,536 and 2,068 for the Commonwealth and 3,750 and 1,756 for foreign countries. Distribution of full-time students among the faculties showed no significant change, but the proportion in pure science increased from 21·3 to 22·2 per cent, in technology from 13·3 to 13·9 per cent, and that in medicine decreased from 15·7 to 14·4 per cent. Full-time advanced students of pure science numbered 3,675 (35 per cent); of technology 1,650 (15·7 per cent); and medicine and dentistry 1,041 (9·9 per cent); 2,870 students were working for the teacher's diploma. Of the full-time students, 71,713 were reading for a first degree, 3,969 for a first diploma, and 13,379 engaged in research or other advanced work, the corresponding figures for 1955–56 being 67,850, 4,021 and 12,668.

The proportion of assisted students was 75·7 per cent compared with 74·9 per cent in 1955–56 and 71·9 per cent in 1953–54, ranging from 90·4 per cent in Wales, 83·8 per cent for English universities and university colleges, excluding Oxford, Cambridge and London, to 61·7 per cent for Scotland. Of the recurrent income of £41,635,609 (an increase of £3,338,958 on 1955–56), £29,082,526 was from parliamentary grants (69·8 per cent). Income from fees decreased from 11·2 to 10·8 per cent, local authority grants increased from 2·9 to 3·1 per cent, payments for research from 6·0 to 6·5 per cent, gifts and subscriptions from 1·0 to 1·2 per cent, and endowments were again 4·0 per cent. Full-time teaching staff increased to 10,485, compared with 10,202 in 1955–56 and 9,810 in 1954–55.

The proportion of full-time students residing in colleges and halls of residence was 27·4 per cent, compared with 27·5 in 1955–56, but the total, 24,652, was higher. The proportion of men in residence was 23·2 per cent and of women 39·9 per cent; whereas 41,291 (46·0 per cent) were in lodgings and 23,923 (26·6 per cent) at home, compared with 44·7 per cent

* University Grants Committee. Returns from Universities and University Colleges in receipt of Treasury Grant, Academic Year 1956–1957. Pp. 50. (Cmnd. 477.) (London: H.M. Stationery Office, 1958.) 4s. net.

and 27·8 per cent, respectively, in the previous year. Non-recurrent grants in respect of capital expenditure totalled £9,134,185, compared with £7,084,366 in 1955–56, and of the recurrent expenditure of £41,509,703—an increase of 9·1 per cent on 1955–56—7·0 per cent was spent on administration, 68·4 per cent on departmental maintenance, and 12·9 per cent on maintenance of premises. The largest increases were in rates, insurance, heat, light, water, wages, etc. (15·3 per cent), departmental wages (technicians and laboratory assistants) (14 per cent), and in repairs and maintenance of buildings (13·9 per cent). Expenditure on libraries (excluding general maintenance, rates, etc.) increased from £1,468,512 to £1,620,958 but remained at 3·9 per cent of the total.

SCIENTIFIC FILMS AT THE BRITISH ASSOCIATION

FOR the fourth successive year the Scientific and Technical Group of the Royal Photographic Society of Great Britain, in conjunction with the Scientific Films Committee of the British Association, presented a series of research films as part of the annual meeting of the British Association. These films were shown in two sessions during the morning and afternoon of August 30. The morning session was under the chairmanship of Sir Raymond Priestley, and during the afternoon Mr. Bowler, vice-president of the Society, took the chair.

Several films were devoted to biological subjects, from the study of the mating behaviour of marsupial frogs to the investigation of cancerous cells. The muscle structure of sea-anemones, courtship of *Drosophila melanogaster*, nematodes and *Leishmania donovani* were also objects of investigation. In what might be described as the industrial field many problems have been covered with ciné techniques, including the casting of steel, coke-oven performance, vehicle suspension systems, flooring research, bookbinding operations and the flow of water in large pipes.

From the psychological aspect, two films were shown which demonstrated techniques that have been employed to identify the particular point of a general scene at which a subject is looking, thus enabling eye co-ordination to be studied. Also in this field an extract was shown from a film which studied parent/child relationship in an experimental treatment of the problems involved in admission to hospital of children under school age.

In pure physics, the motion of electrons in electric fields was demonstrated by means of charged droplets; this film was made in Germany. Automatic self-replication was also shown by ingenious systems of models. Finally, Mr. Hallett, secretary of the Society, gave a short talk on the scope and aims of the "Encyclopædia Cinematographica", with examples of research films loaned by Dr. Wolf, of Göttingen.

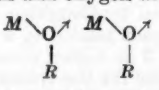
Among the methods used to prepare these films were time-lapse and high-speed ciné-photography, interference and phase-contrast microscopy, electron microscopy and television techniques. Colour and monochrome, silent and sound-track films were all represented at this meeting, which admirably demonstrated the wide variety of ways in which photography can be of use in research in an equally wide range of scientific investigation.

A more detailed report will be published in the *Journal of Photographic Science*.

A STRUCTURAL THEORY FOR METAL ALKOXIDE POLYMERS

By Dr. D. C. BRADLEY
Department of Chemistry, Birkbeck College, University of London

RECENT studies[1,2] on the metal alkoxides $M(OR)_n$, where M is an n-valent metal and R is an alkyl group, have established that derivatives with the lower primary or secondary alkyl groups are in fact polymeric, that is $\{M(OR)_n\}_x$, where x is the degree of polymerization. It is believed that this polymerization is caused by intermolecular bonds involving the metal and oxygen atoms, namely:



and is a manifestation of the tendency of the metal to expand its covalency by co-ordination with oxygen. It is a striking feature of the metal alkoxides that the alternative mechanism for this covalency expansion, namely, co-ordination between the alkoxide and a ligand containing oxygen or nitrogen (for example, $M(OR)_n,xL$), is relatively rare and this suggests a special stability for the polymer. Moreover, it is a general rule that, in a series of metal alkoxides with metals of the same sub-group (for example, titanium, zirconium, thorium) and having the same alkyl groups, the degree of polymerization x increases with the size of the metal atom. Nevertheless, only in a few cases have structures been suggested for metal alkoxide polymers[3–7]. Another interesting feature of these compounds is that the polymers are relatively small compared with typical organic polymers and silicones. Recent studies on the hydrolysis of titanium alkoxides[8,9] showed that the titanium oxide alkoxides $TiO_h(OR)_{4-2h}$ formed were also low polymers, and this behaviour was explained in terms of a series of structural models in which titanium was 6-co-ordinated by oxygen. Further consideration of these structures reveals that in each case the degree of polymerization is unique in being the lowest possible, and this led me to explore the possibility of rationalizing the polymeric nature of metal alkoxides in terms of the stereochemistry of the metal. It is the purpose of this article to show that a rationalization can indeed be achieved on the basis of the following single new major assumption. It is assumed that the metal alkoxide adopts the smallest possible structural unit consistent with all the metal atoms attaining a higher co-ordination number. Also the choice of structure is limited by the condition that the co-ordination number of oxygen does not exceed

Table 1

Compound	Co-ordination No. of *M*	Stereochemistry of *M*	Minimum degree of polymerization
MOR	2	*OMO* = 90°	2
	2	*OMO* = 120°	3
	3	Pyramidal	4*
$M(OR)_2$	3	Planar, one *OMO* = 90°	2
	3	Planar, *OMO* = 120°	3
	4	Tetrahedral	3
	4	Planar	4
	6	Octahedral	Infinite 3-dimensional polymer
$M(OR)_3$	4	Tetrahedral	2*
	4	Planar	2
	6	Octahedral	8*
$M(OR)_4$	5	Trigonal bipyramidal	2
	6	Octahedral	3*
	8	Cubic	8
$M(OR)_5$	6	Octahedral	2*
	8	Cubic	4
$M(OR)_6$	8	Cubic	2*

* Agrees exactly with predictions of the theory

four. Then the degree of polymerization can be predicted in terms of the stereochemistry of the metal. Some typical deductions from this theory are given in Table 1 and it is clear that in general the polymers predicted are very low ones. Next we shall see how far the experimental evidence supports this theory. Unfortunately there are to date no direct structural determinations available on metal alkoxides and we can only compare the predicted degree of polymerization with that determined cryoscopically or ebullioscopically, bearing in mind that these experimental values are averages.

Univalent Metal Alkoxides

Sidgwick and Sutton[3] found that thallous alkoxides were tetrameric and proposed a structure in which each thallium atom was bonded pyramidally to three oxygens and each oxygen was bonded to three thalliums and one alkyl group. This agrees exactly with the above theoretical considerations. No other data are available on univalent metal alkoxides. The alkali metal alkoxides are usually assumed to be ionic, but in view of the covalent nature of alkali metal zirconium ennea-alkoxides[10] there is the possibility that the alkali metals, especially lithium, may form covalent alkoxides. Similarly, it will be worth while to investigate cuprous, argentous and aurous alkoxides.

Bivalent Metal Alkoxides

No data are available on bivalent metal alkoxides although some have been prepared. The case of beryllium is particularly interesting because it is expected to adopt a tetrahedral configuration with oxygen and should thus form trimeric dialkoxides with the structure already proposed[3]. On the other hand, 4-co-ordinated bivalent copper might adopt the planar configuration resulting in cyclic tetrameric cupric alkoxides. A cyclic trimer is unlikely because this would bring the copper atoms too close together.

Tervalent Metal Alkoxides

Aluminium alkoxides are well known and some molecular weights have been determined[4,11,12]. Robinson and Peak[4] proposed a structure for the tetrameric alkoxides involving a cyclic system with 4-co-ordinated aluminium. However, the above theory predicts a dimer for 4-co-ordinated tetrahedral aluminium and there is no obvious reason why a cyclic tetramer should obtain. On the other hand, it is known that aluminium can achieve a maximum co-ordination number of six and for octahedral aluminium the theory predicts an octamer. Thus the tetramers could result from a distribution of the aluminium between 4- and 6-co-ordination.

In fact, the possibility of two higher co-ordination numbers for aluminium is probably the cause of the unusual behaviour of aluminium trialkoxides. For example, Mehrotra[13] has demonstrated an 'ageing effect' in which the degree of polymerization increases with time. Also, a hysteresis effect has been detected in measuring the vapour pressures of aluminium alkoxides[13]. In addition, Mehrotra[13] found that aluminium *iso*propoxide was dimeric in the vapour state. These facts can readily be interpreted on the basis of the structural theory. Assuming that the freshly distilled or sublimed alkoxide is initially dimeric and contains tetrahedral aluminium, then the ageing effect will be due to a structural rearrangement as the aluminium realizes its maximum co-ordination number. Clearly, this rearrangement in a solid at room temperature will be slow and consequently the degree of polymerization of an aluminium alkoxide will depend on its previous history. This could explain the high values reported by Ulich and Nespital[11]. Although the theory predicts the dimer and the octamer for 4- and 6-co-ordinated aluminium, respectively, other unique structures are predicted for systems involving both higher co-ordination numbers. For example, if 75 per cent is 4-co-ordinate and 25 per cent 6-co-ordinate, a tetramer is possible containing a central octahedral aluminium attached through alkoxide bridges to the three tetrahedral aluminiums[3]. This corresponds to the completion of the first distinct stage in the ageing process and it is extremely interesting to note that Robinson and Peak and Mehrotra found tetrameric aluminium alkoxides. The remaining unique structure is a hexamer involving 66·7 per cent 4-co-ordinate and 33·3 per cent 6-co-ordinate aluminium. In this structure[3] the molecule contains two 6-octahedral aluminiums joined through an alkoxide bridge and each one is attached through alkoxide bridges to two tetrahedral aluminiums. The second stage in the ageing process should then result in the formation of this hexamer and the final stage would involve the formation of octamers in which the aluminium is entirely 6-co-ordinated.

Quadrivalent Metal Alkoxides

Titanium tetra-alkoxides have been known for many years, but the first results of structural significance were reported by Caughlan *et al.*[5], who found that these compounds tended to a limiting degree of polymerization of 3 at higher concentrations. They proposed a structure for the trimer (Fig. 1) based on 6-co-ordinated titanium with three octahedra joined together through the sharing of common faces. As can be seen from Table 1, this trimeric structure is in accordance with the predictions of the theory. Even more striking examples of structures which are logical consequences of this theory are the titanium oxide ethoxides $Ti_6O_4(OEt)_{16}$ (Fig. 2) and[6] $Ti_{12}O_{12}(OEt)_{24}$. Although titanium appears limited to a maximum co-ordination number of 6, zirconium, cerium-IV and thorium can achieve a higher co-ordination number of 8. The lowest possible polymer for a tetra-alkoxide containing 8-co-ordinated metal is the octamer involv-

ing a cyclic configuration of body-centred cubes (Fig. 3)[14]. The cube was chosen for 8-co-ordination because there are four oxygens on each face and this gives the maximum degree of sharing of alkoxide groups between any two adjacent metal atoms and hence the minimum degree of polymerization. Zirconium normal alkoxides[6] had an average degree of polymerization of 3·5, which is near the predicted value for 6-co-ordinated zirconium. Moreover, zirconium tetra-*iso*propoxide is trimeric and it forms a solvate $Zr(OPr^i)_4.Pr^iOH$, which is dimeric. A structure was proposed for the solvate[6] involving octahedrally 6-co-ordinated zirconium (Fig. 4) and it is interesting to note that this is exactly in accordance with the structural theory. The lower alkoxides of thorium were insoluble, thus precluding molecular weight determinations, but the longer chain normal alkoxides were soluble and exhibited an average degree of polymerization of 6·3, which suggests that the thorium is predominantly 8-co-ordinated. In view of the availability of two higher co-ordination numbers for zirconium, cerium-IV and thorium, it would not be surprising to find ageing effects in their alkoxides (cf. aluminium).

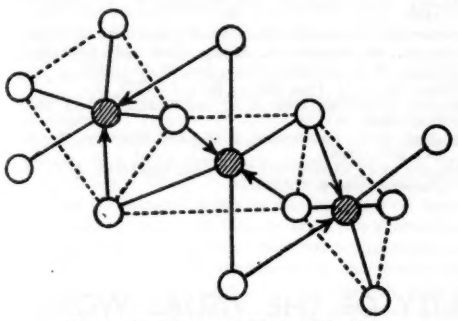


Fig. 1. $Ti_3(OEt)_{12}$. ●, Ti; ○, O in O*Et*

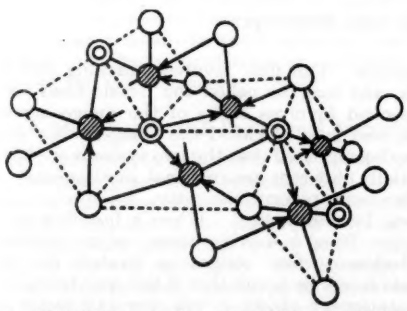


Fig. 2. $Ti_4O_4(OEt)_{10}$. ●, Ti; ○, O in O*Et*; ◎, O in Ti·O·Ti

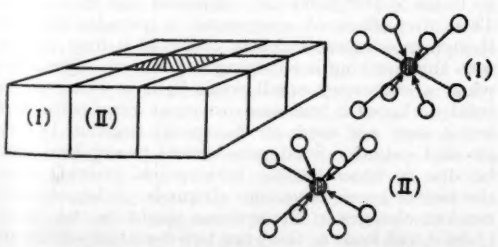


Fig. 3. Structure of $M_3(OR)_{12}$. ●, *M*; ○, O in O*R*

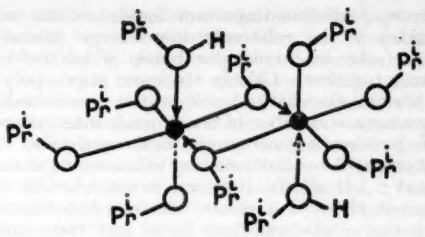


Fig. 4. ●, Zirconium; ○, Oxygen

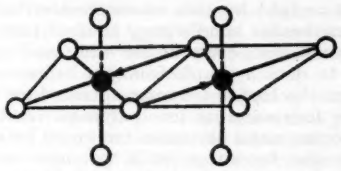


Fig. 5. ●, Tantalum; ○, Oxygen

Quinquevalent Metal Alkoxides

It can be seen from Table 1 that a penta-alkoxide containing an octahedrally 6-co-ordinated metal should be dimeric. Recent work on normal alkoxides of niobium[15] and tantalum[7] showed that they were dimeric and a structure was proposed for the tantalum derivatives in agreement with the theory (Fig. 5). Moreover, a number of uranium penta-alkoxides were also found to be dimeric but the penta methoxide $U(OMe)_5$ was trimeric and it was suggested that in the latter there might be a significant proportion of 8-co-ordinated uranium[15]. Thus the smallest polymer for a penta-alkoxide containing an 8-co-ordinated metal with the cubic configuration would be the tetramer. A tetramer will also result from a square antiprism configuration.

Sexavalent Metal Alkoxides

The theory predicts a dimeric state for a hexa-alkoxide containing an 8-co-ordinated metal with either the cubic body-centred or square antiprism configuration. Measurements on uranium hexa-methoxide $U(OMe)_6$ show that it is predominantly dimeric (Bradley, D. C., and Chatterjee, A. K., unpublished work), suggesting that the uranium is 8-co-ordinated.

This survey of experimental data has shown several examples (Table 1) which agree exactly with the predictions of the theory and other cases where the known degree of polymerization is within the limits set by the theory (that is, where more than one higher co-ordination number is available). The variety of examples which fit the theory make it unlikely that the agreement is merely coincidental. Therefore it is interesting to consider why metal alkoxides adopt the structures with the lowest degrees of polymerization and to discover whether this phenomenon is shared with other types of compound. Undoubtedly a factor of fundamental importance is the covalency expansion of the metal, since this causes the polymerization. Thus in the formation of organic high polymers, no change in covalency of carbon is involved, and similarly in the silicones the silicon is essentially quadricovalent although some π-bonding involving 3*d* orbitals of the silicon may contribute to the

structure. Another important feature of the metal alkoxides is the relatively low energy (about 10 kcal.) of the intermolecular bonds which hold the polymer together. Clearly the most stable polymer (to thermal dissociation) will be the one containing the maximum number of these weak intermolecular bonds between adjacent units in the polymer. This point can well be illustrated by reference to titanium alkoxides. If all the titanium is octahedrally 6-co-ordinated there is a choice between the trimer in which the octahedra share faces and each unit is held by three intermolecular bonds, and a higher polymer in which the octahedra share edges and each unit is held by two intermolecular bonds. If the intermolecular bond energy is approximately the same in each case, then 50 per cent more energy is required to detach a unit from the trimer molecule than from the higher polymer. According to these ideas the formation of low polymers need not be limited to the metal alkoxides but could be expected wherever the formation of a polymer is due to covalency expansion of an element and wherein the intermolecular bonds are weak. For example, some of the electron-deficient molecules such as the boron hydrides and metal alkyls support this view. Diborane is formed from BH_3 because the tercovalent boron increases its covalency to four. This covalency expansion could be achieved in an infinite linear polymer $(BH_3)_\infty$ in which each unit of the polymer is held by only one hydrogen bridge as against the two in diborane. However, it must be realized that in some systems of inorganic polymers (for example, borazoles and phosphonitrilic compounds) also involving covalency expansion there are other complicating factors such as π-bonding and aromaticity which may stabilize particular structures, and it does not follow that the molecule will adopt the lowest possible degree of polymerization.

It is hoped to test the theory discussed above by direct structural measurements on the metal alkoxides. However, it is always possible that in the crystal lattice a metal alkoxide may adopt a degree of polymerization different from that exhibited in solution.

I am indebted to the Chemical Society for permission to reproduce the diagrams in Figs. 1–5, and also to Prof. W. Wardlaw for his guidance and encouragement throughout this work.

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EYE MOVEMENTS AND THE STABILITY OF THE VISUAL WORLD

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THE visual perception of movement is generally regarded as of primary biological importance, yet far less work has been done on this subject than on topics such as colour vision, visual acuity and the photic sensitivity of the eye. Further, surprisingly little is reliably known about the neurological mechanisms subserving movement perception. There are many unsettled questions, in particular that of the apparent stability of the visual world during voluntary movements of the eye. Some of the problems raised by the visual perception of movement will be discussed in this article.

Information Channels in the Perception of Movement

The simplest case of movement perception is that given by a moving self-luminous object in a dark room. Perception of its movement may occur under two different sets of conditions: (1) the eyes may follow the object ('tracking'), in which case its image will, ideally, remain stationary upon the retinæ; and (2) the eyes may remain stationary so that the images travel across the retinæ. Since movement is experienced under both conditions, there must be at least two information channels subserving movement perception. The first involves tracking eye movements, and may be called the 'retina/head' system. The second involves shifts of the image across the retina, and may be called the 'image/retina' system. It may be supposed that the two systems are based on essentially different neurological mechanisms.

Movement perceptions may, in special circumstances, be paradoxical. When a fast-flowing river, the view from a moving train, or an endless belt of black-and-white stripes is fixated for several seconds it will be found that if the gaze is transferred to a stationary object it will seem to move in the opposite direction and with corresponding velocities to those of the previously observed real movement. This after-effect of movement is paradoxical, for though movement is clearly observed it may also be seen that nothing is changing in position. Similarly, when a stationary small weak light is observed in total darkness it will seem to move erratically, and yet it may not seem to change its position to any marked extent. Such paradoxical perceptions may be due to incompatible information arriving from the two or more movement channels. Adaptation or random changes in the systems might be the cause.

As is well known, there are two essentially different types of eye movement, first studied systematically by

Dodge[1,2]. When the eye tracks a moving target, once it is 'locked' on to the target it moves smoothly, provided that the target moves in a simple, known manner without excessive changes in direction or velocity. Voluntary movements in the absence of a target take place in small jerks ('saccades') occurring about four times per second, which have been much studied particularly in relation to reading.

It is generally supposed that smooth tracking movements of the eyes occur only in response to retinal cues, that is they form part of what we have called the 'retina/head' system. I have shown that this is not necessarily the case. A small neon lamp is arranged to flash at 25–50 flashes/sec. : the light will appear continuous under conditions of steady fixation, but if, with the eyes remaining still, the lamp is moved steadily across the retina, a regular flicker will be noticed. This is a well-known effect. If now the eye is swept as smoothly as possible by voluntary movement, this regular flicker is not seen. The lamp seems to move in jerks. Evidently the saccadic movements prevent the smooth travel of the image which is necessary for the observation of the regular flicker. On the other hand, when the eyes follow a moving target the flicker may be observed, as when the lamp is moved across the field with the eyes stationary. Using this technique, I have been able to show that it is not necessary for the observer to track a visual object for the smooth eye movements to occur. The regular flicker indicating smooth eye movements may be seen if the observer tries to track his own hand under conditions of complete darkness apart from the light from the neon lamp, his hand being invisible. That is to say, smooth eye movements may be elicited even in the absence of appropriate retinal stimuli. It follows that the retina/head system may involve a proprioceptive component ; information from moving members of the body may suffice to actuate the smooth eye movement system. I have also found that the apparent movements of a stationary small weak light viewed in darkness (the 'autokinetic effect') are influenced by slight unbalanced strains imposed on the eye or neck musculature. The effect may normally be due to randomly occurring imbalance of the musculature or proprioceptive systems of the eye and neck.

Eye Movements and Perceptual Stability

It is a commonplace to state that when the eyes move voluntarily across an extended field (by means of saccadic movements) there is little, if any, change in apparent position of the perceived objects. The visual world does not swing round in the direction opposed to the eye movement. On the other hand, nearly all subjects report that when an object moving across the field is tracked, the stationary background does tend to swing round in the opposed direction. The experience is similar to, though probably less marked than, the movement of the field observed when the eye-ball is moved passively by pressure from the finger. This observation suggests that saccadic eye movements are in some way necessary for the stability of the visual world. It may be that the jerky movements of the eye are themselves important, or that the saccadic mechanism accepts information giving rise to stability which is rejected during smooth tracking movements. Evidence to be given below suggests that the former is the case.

The problem of the stability of the visual world was discussed by Helmholtz[3], and later by many other writers, the most recent being Mackay[4]. There have been two main theories. The first, that stabilization is given by proprioceptive information from the extrinsic eye muscles, which has been called the 'inflow' theory, was rejected by Helmholtz, who held that the central command signals (but called by him 'the Will') are monitored by an internal loop which gives appropriate information for providing stability, provided the motor system is functional. This has been called the 'outflow' theory. He pointed out that patients suffering from paralysis of the external recti, and who were therefore unable to execute voluntary eye movements, experience the visual world moving round in the direction in which the eyes should have moved had it been possible to "execute the command". This observation has been confirmed under more controlled conditions[5].

A useful technique for investigating many of the phenomena of stabilization and movement perception is provided by observations of after-images (preferably produced by fixating a photographic-type electronic flash tube) under various conditions. It is not always realized that after-images observed in complete darkness move, apparently rather precisely, with the eyes during slow voluntary eye movements though they are not affected by passive movements. We should expect some feed-back from the stretch receptors which are now known to exist in the eye muscles[6,7] during passive movements, and this should produce some shift of after-images if the inflow theory were true.

We find that if, in total darkness, the drift of an after-image is observed during (*a*) slow voluntary eye movements, and (*b*) during the 'proprioceptive tracking' of the observer's hand described above, the after-image shows small saccadic-like jerks in condition (*a*), but not in condition (*b*), when it moves smoothly. If the velocity of the hand movement is changed the after-image may seem to lead or lag, and then to 'lock' on to the proprioceptive locus of the hand. The jerks experienced in condition (*a*) suggest that the stabilizing system has access to information of saccadic movements.

The Cancellation Theory

Helmholtz does not clearly state how his outflowing command signals are supposed to produce stability. Since stability depends upon the eyes in fact moving, the shift of the retinal image must be important. The term 'outflow' theory is thus misleading, for it ignores this shift of the retinal image and the information it transmits. Passive eye movements produce marked contrary shifts of the visual world : Helmholtz's theory requires these movement signals in conjunction with his command signals to produce stabilization, and this should be stated explicitly. The 'outflow' theory may, in this form, be called the 'cancellation' theory, where signals from the eye/head system are supposed to be cancelled out by signals from the image/retina system. This is similar to the view put forward by von Holst[8], but is really a natural extension of Helmholtz's theory.

Mackay[4] has recently made the interesting suggestion that stabilization is not something which is achieved, but rather that things are regarded as stable unless there is sufficient evidence to the contrary. Thus he says : "what requires informational justification is not the maintenance of stability but the perception of change", and also : "the retinal changes resulting from voluntary movement evoke no

perception of world-motion, because they are not an awkward consequence to be compensated, but part of the goal to be achieved". He explains the apparent movement of the perceptual world when the eye movements are paralysed by saying that the 'goal' (which is internal and conceptual) shifts with changing expectations. This may be contrasted with the cancellation of the command signals by retinal movement information.

On the 'cancellation' theory the world moves if the eye movement is thwarted because the command signal is not cancelled by the image/retina system, there being no signal. Similarly, after-images move during voluntary eye movements, because there is no shift of the image across the retina to give an image/retina signal.

On the 'cancellation' theory we may predict that if we could destroy movement information in the image/retina system then the visual world should seem to move with the eye during voluntary eye movements. This may be done simply by illuminating the field with a stroboscope set to 4–5 flashes/sec. Movement is then only seen when the eye follows an object. With the eyes stationary, a moving object is observed to occupy successive positions, but not to move. When it is tracked it does, however, in stroboscopic illumination, appear to move. Voluntary eye movements produce loss of stability; the entire visual field moves in jerks. If now smooth eye movements are produced, by tracking a small self-luminous object held rather close to the eye, the entire visual world may be seen to move with the tracking object, and therefore with the eyes. This is observed best with a faintly glowing filament mounted on a pendulum bob; the visual world then swings with the bob, provided the distance and velocity conditions are appropriate.

This is difficult to reconcile with Mackay's 'null hypothesis' view of the matter, for the expectation of movement can scarcely be supposed to be related to the manner of illumination or to the kind of movement of the eye.

Mackay's observation that a stationary glowing filament moves though the stroboscopically lit field remains relatively stable during passive eye movements would be expected on the 'cancellation' hypothesis, for the image/retina system should give movement information from the self-luminous filament only, and the eye/head system will give no information. The new observation given here, that the visual world moves with the eyes under these conditions when following a target, seems to be positive evidence for the cancellation hypothesis.

It may be suggested that saccadic movements are important in providing large movement signals which may be readily computed to give cancellation.

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BETA-PROPIOLACTONE AS A TOXOIDING AGENT

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THE conversion of a bacterial exotoxin into a non-toxic antigen suitable for injection into animals presents special difficulties in the case of an unstable substance such as the β-toxin of *Cl. welchii* Types *B* and *C*. This antigen, which is used for vaccinating domestic animals against enterotoxæmia, deteriorates rapidly[1,2], even at 4° C.

The usual method of toxoiding β-toxin for vaccine preparation involves incubating the culture filtrates with formalin at 37° C. until the material is no longer lethal for mice. Culture filtrates with initial minimum mouse lethal doses of 0·005–0·001 ml. normally require 9–21 days to become non-toxic. More concentrated solutions of β-toxin, prepared by ammonium sulphate precipitation or by freeze-drying of the culture filtrates, take proportionately longer.

The antigenic potency of these vaccines is tested in rabbits as follows: two doses of 2 ml. each of the inactivated preparations to which alum is added to a final concentration of 1·0 per cent are injected. There is an interval of four weeks between the two injections and the rabbits are bled two weeks later. The antibody contents of sera obtained in this manner from about ninety rabbits used for testing twelve batches of formalin-inactivated culture filtrate averaged 7·58 international β-antitoxin units/ml. Seventeen batches of formalin-inactivated Type *B* culture were tested by the procedure recommended in the British Veterinary Codex for Lamb Dysentery Vaccine and the antibody contents from the rabbit sera averaged 2·75 international β-antitoxin units/ml. (the minimal response for this vaccine should be 2·5 international β-antitoxin units/ml.).

A concentrated Type *C* filtrate (minimum mouse lethal dose, 0·0002 ml.) was used for comparing the toxoiding properties of β-propiolactone

with that of formalin. The final concentrations of β-propiolactone used were 0·3, 0·6, 0·9 and 1·0 per cent (v/v); formalin concentrations were 0·6 and 3·0 per cent. The β-propiolactone was added as a chilled, freshly prepared 10 per cent (v/v) aqueous solution and the mixtures were buffered with 0·5 *M* disodium hydrogen phosphate. Formalin was added as a 40 per cent solution of formaldehyde without buffering. The reactions with β-propiolactone were complete after 2 hr. incubation at 37° C., when no more acid decomposition products were formed. The mixtures treated with 0·3 and 0·6 per cent β-propiolactone were still lethal to mice, but when pooled were tolerated by rabbits, with, however, some œdema. The portion of filtrate treated with 0·6 per cent formalin was still highly toxic after 8 days incubation at 37° C. and was discarded. The portion treated with 3·0 per cent formalin became non-toxic after 3 days.

The antigenicity of all the samples was tested by the procedure already described, using three rabbits for each of the β-propiolactone-treated samples and two rabbits for the formalin-treated one. The antibody response of these rabbits is shown in Table 1.

Table 1. ANTIBODY RESPONSES TO β-TOXIN TOXOIDED WITH β-PROPIOLACTONE OR FORMALIN

Treatment of β-toxin preparations	Antibody contents of rabbit sera (β-antitoxin units/ml.)
0·3 and 0·6 per cent β-propiolactone (pooled samples)	100 60 170
0·9 per cent β-propiolactone	120 50 50
1·0 per cent β-propiolactone	80 80 110
3·0 per cent formalin	4 20
0·6 per cent formalin (12 different culture filtrates)	7·58 (mean value from sera of 90 rabbits. Range 22–3)

The reactions of β-toxin, before and after treatment with β-propiolactone, with a homologous horse antiserum in agar gel are shown in Fig. 1. The continuity of the band of precipitate formed between the antiserum and the various antigens in the peripheral wells shows that the reduction in toxicity obtained by treatment with β-propiolactone was not accompanied by any alteration in the reaction of the toxoid with antibody against the original toxin.

A similar experiment was undertaken with a purified preparation of β-toxin, a more stable protein antigen[3]

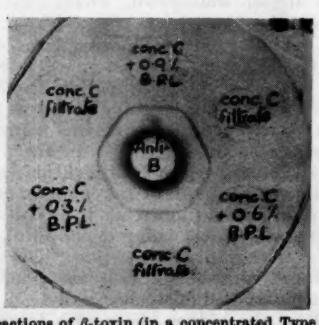

Fig. 1. Reactions of β-toxin (in a concentrated Type *C* filtrate) before and after treatment with β-propiolactone (*B.P.L.*), with Type *B* antiserum

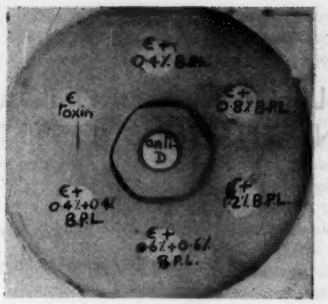

Fig. 2. Reactions of ε-toxin before and after treatment with β-propiolactone (*B.P.L.*), with Type *D* antiserum

prepared from filtrates of *Cl. welchii* Type *D*. The toxic and immunizing properties of β-propiolactone- and formalin-treated samples were examined together with their precipitation in agar gel and flocculation by the optimum proportions method. Here again, it was found that the toxicity was reduced much more rapidly by β-propiolactone than by formalin; that, in agar gel using the homologous horse antiserum, a common band of precipitate was given by the untreated samples and those treated with β-propiolactone at concentrations up to 1·2 per cent (Fig. 2). Samples treated with less than 0·9 per cent β-propiolactone had the same flocculation rates and optima as the untreated samples; higher concentrations of β-propiolactone led to a shift of the optimum towards higher antigen concentrations. In contrast to this, formalin treatment that was just sufficient to render our ε-antigen non-toxic produced a toxoid that no longer gave recognizable flocculation zones with antiserum and the reaction of which in agar gel was markedly altered, as was also shown by Schuchardt, Munoz and Verwey[4]. However, the antibody responses of rabbits inoculated with a β-propiolactone-treated ε-toxoid were only slightly better than those obtained with a similar formalin-treated preparation.

The rapidity of the interaction of β-propiolactone with protein antigen makes it possible to avoid the prolonged incubation at 37° C. required by the formalin toxoiding process at the usual formalin concentrations, and any deterioration resulting from this. The apparent lack of change in the flocculating and agar gel precipitation behaviour of the β-propiolactone-treated toxoids indicates that β-propiolactone does not produce the changes in solubility and other side reactions (for example, cross-linking) which occur with formaldehyde. The experiments with ε-toxin, however, have shown that the preservation of these *in vitro* properties is not necessarily an index of better immunizing properties. This was also shown by Schuchardt *et al.*[4], who found that formalin-toxoided ε-preparations which no longer gave visible reactions with antitoxin still produced high antitoxin titres in sheep. The tenfold improvement in antibody response found when β-toxin is toxoided with β-propiolactone rather than formalin may be due to the inhibition of the breakdown process, probably enzymatic, which is responsible for the quick deterioration of β-toxin.

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ROLE OF HYALURONIDASE IN THE RE-ABSORPTION OF WATER IN RENAL TUBULES: THE MECHANISM OF ACTION OF THE ANTIDIURETIC HORMONE

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ACCORDING to recent theories the antidiuretic hormone acts by increasing the permeability of the nephron walls. As a result, hypotonic urine, following the osmotic gradient, is reabsorbed[1-3]. That the neurohypophysial hormone dilates the minute membrane pores has been demonstrated for frog skin[4]. Diuresis and antidiuresis are presumed to be due to a similar change of pore size in the membranes of the distal tubules. Yet there is no direct evidence for this suggestion; it is based on indirect information, deduction and the analogy with the tests on frog skin.

A few years ago we discovered that under certain conditions the urine of all mammals contains hyaluronidase[5]. Hyaluronidase activity depends on the extent of diuresis, dropping to zero after water loading (Fig. 1).

A special analysis has shown that the reduction of activity during high diuresis cannot be accounted for simply by dilution of the enzyme entering the nephron lumen at a fixed concentration. The experiment in which increased urine formation is effected by osmotic diuretic loading, and not by water loading, is of particular significance. As is seen from Fig. 2, the increased urine flow during osmotic diuresis is not accompanied by decreasing hyaluronidase activity.

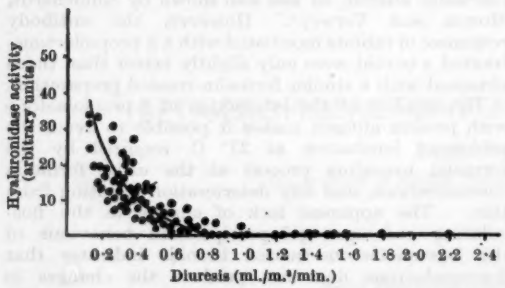

Fig. 1. Variation of diuresis with hyaluronidase activity

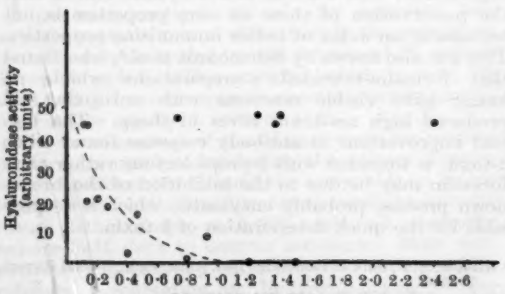

Fig. 2. ●, Osmotic diuresis; ○, water diuresis. According to experiments on the same dog

Hence we can infer that the amount of hyaluronidase in the nephron lumen is increased by neurohypophysial hormone: as the secretion of antidiuretic hormone is inhibited (water diuresis) the enzyme content in the urine drops, but it remains high when the hormone acts on the kidney (osmotic diuresis). This suggestion is supported by the following experiment.

Urea was administered to rats during complete water diuresis. As a result, urine formation increased. Pituitrin was then injected and diuresis decreased somewhat, but it remained high. Nevertheless, as is seen from Table 1, the urine acquired a considerable hyaluronidase activity[6].

Table 1

Water loading		Administration of urea		Injection of pituitrin	
diuresis (ml./mm.)	activity (arbitrary units)	diuresis (ml./mm.)	activity (arbitrary units)	diuresis (ml./mm.)	activity (arbitrary units)
1·2	0	2·5	0	1·9	11
1·5	0	5·5	0	2·2	13
1·8	0	7·0	0	1·1	11
2·8	0	6·0	0	5·5	28
2·8	0	6·0	0	2·8	31
4·5	0	6·0	0	2·8	30
4·5	0	11·0	0	2·8	16

These results led us to the conclusion that in the course of facultative re-absorption, osmotically free water leaves the lumen of the distal tubules not through the cell cytoplasm but through the intercellular cement, with hyaluronic acid as a constituent. In a state of hyperhydration of the organism, this cement is almost waterproof, which enables hypotonic urine to be formed.

During dehydration, antidiuretic hormone causes hyaluronidase secretion by the nephron cells. Under the action of the enzyme, mucopolysaccharides of the intercellular cement are depolymerized and undergo hydrolysis, the multicellular tubule membrane becoming permeable to water. By means of a direct histochemical study we have verified this suggestion[7].

White rats were used in our experiments. One group of animals was killed at the peak of water-diuresis, the other after being deprived of water for 48 hours or after pituitrin injection. The kidneys of hyperhydrated and dehydrated rats were quickly removed and studied histochemically.

On staining the kidney slices with toluidine blue, we found, in the area of the collecting ducts, structures

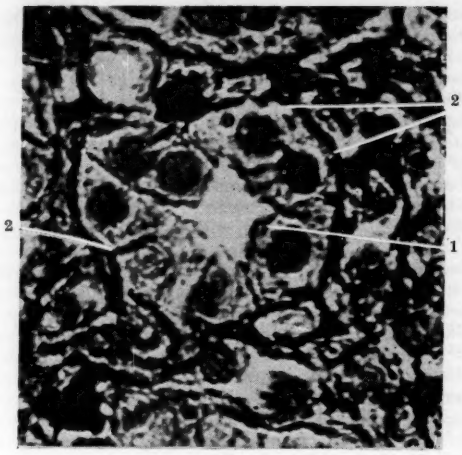

Fig. 3. The collecting duct region during water diuresis. 1, duct epithelium; 2, mucopolysaccharides in the intercellular cement and in the basement membrane; these are black on the microphotograph and stained red on the slice. (× 1,080)

showing the typical red metachromasia characteristic of hyaluronic acid. At the peak of water diuresis, the collecting duct epithelium consists of highly prismatic cells, with abundant cytoplasm and nuclei in the centre of cells (Fig. 3). Epithelial elements are cemented by intercellular layers stained red, which shows that they contain hyaluronic acid. On the photomicrograph they are seen as black lines. The basement membrane reveals a similar metachromasia which appears as clearly outlined fibres (red in the preparation and black in the photomicrograph) surrounding every duct.

This picture, typical of the kidney of a hydrated rat, changes abruptly when the animal undergoes dehydration or when pituitrin is administered (Fig. 4). The cells lose most of their cytoplasm and the nuclei project into the lumen. The reason for this change in the epithelium is the complete dissolution

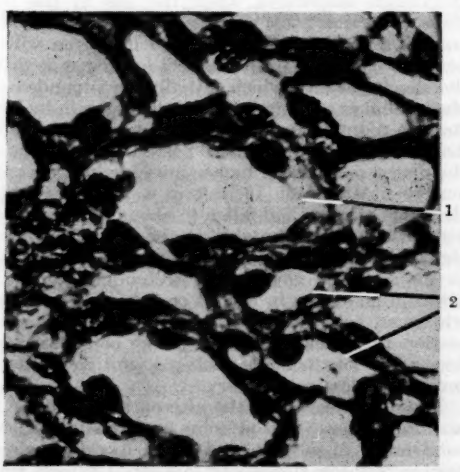

Fig. 4. The same region as Fig. 3 after pituitrin injection. The flattening of the duct epithelium is shown. Positive reaction to γ-metachromasia has given place to a diffuse pinkish background. 1, collecting ducts; 2, lymphatic capillaries. (× 1,080)

of the cytoplasmic apical ends into the lumen of the tubules. This loss of cytoplasm appears to be identical with apocrine secretion of the collecting duct epithelium due to antidiuretic hormone.

As a result of the antidiuretic hormone effect, the metachromasia of the fibres stained by toluidine blue changes, turning violet instead of red. When these changes reach their maximum, the mucopolysaccharide intercellular layers disappear completely and the hyaluronic elements of the basement membrane are no longer seen. γ-Metachromasia remains only as a diffuse homogeneous pinkish background uniformly filling in all the interstices between the ducts.

After injection of pituitrin, numerous tubules, previously unseen, appear in the meshes of the intertubular mucopolysaccharide net in the immediate vicinity of the ducts. In the cross-section of the walls of these new tubules, cells typical of the capillary endothelium are seen. There is much evidence that these tubules are lymphatic kidney capillaries which open under the influence of the antidiuretic hormone.

The processes which occur in the area of the collecting ducts as the result of natural antidiuresis brought about by continued lack of water are, in principle, similar to those that occur after injection of pituitrin.

The rapidity of morphodynamical change in the system of collecting ducts is startling: they reach a maximum 15–20 minutes after the injection of the hormone.

Water administered to dehydrated animals causes a quick reversal of all these changes. The first signs of the regeneration of hyaluronic stroma of the intercellular substance and epithelial elements appear in 20 minutes. Sixty minutes later, when intensive water diuresis sets in, the tubule structure approaches the state characteristic of the hydrated kidney. There is little doubt that the changes in kidney structure which are the morphological expression of antidiuretic hormone action underlie the antidiuretic action of the hormone.

We have arrived at the following conclusions based on these results.

1. Antidiuretic hormone stimulates the collecting duct cells, which begin to secrete apocrine-wise, hyaluronidase being a constituent of the secretion.

2. The secreted enzyme rapidly depolymerizes mucopolysaccharide complexes of the intercellular cement and the basement membrane of the tubules. This makes the structures separating the tubule lumen from the interstitial tissue permeable to water. The hypotonic fluid in the tubules then follows the osmotic gradient and undergoes facultative reabsorption.

3. The water entering the interstitial space is drained by an extensive system of newly opened lymphatic capillaries.

4. All these processes are localized in the most distal part of the nephron. It is the collecting ducts that are the main site of facultative water re-absorption. It seems reasonable to change the name 'collecting ducts' as it does not suggest the importance of the processes occurring inside the ducts.

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LETTERS TO THE EDITORS

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Radio Observations of Hypersonic Shock Waves

A shock tube consists essentially of two sections, one containing a gas at high pressure and separated from the test gas in the other section by means of a diaphragm. A shock wave is produced in the test gas when the diaphragm is suddenly broken. As the initial pressure ratio is increased, the shock front attains a limiting velocity $U \approx \frac{\gamma_1 + 1}{\gamma_2 - 1} a_2$, and the corresponding limiting shock Mach number is U/a_1, where a_1, a_2 are the speeds of sound and γ_1, γ_2, the specific heat ratios (C_p/C_v) of the gases in the test and high-pressure sections respectively. Thus if both sections contained the same monatomic gas for which $\gamma = C_p/C_v = 1{\cdot}67$ the limiting shock Mach number would be approximately 4. Greater shock Mach numbers could be achieved if the speed of sound in the high-pressure section were increased, for example, by using a light gas and by raising its temperature. Thus hydrogen is a suitable driver gas, and it could also be brought to a high temperature by burning oxygen in it. An alternative method of producing a high shock velocity is to introduce an intermediate section into the shock tube[1]. When the first diaphragm is broken, a shock wave is produced in the intermediate section, and the resulting column of hot gas flowing at high speed in this section then acts as the final driver to initiate a faster shock wave in the test section. In a previous communication[2] we described the radio measurement of the velocity of shock waves by means of the Doppler effect. The value of this technique, which provides detailed measurement as the shock wave travels along the tube, is illustrated by its application to the study of methods for producing hypersonic flow.

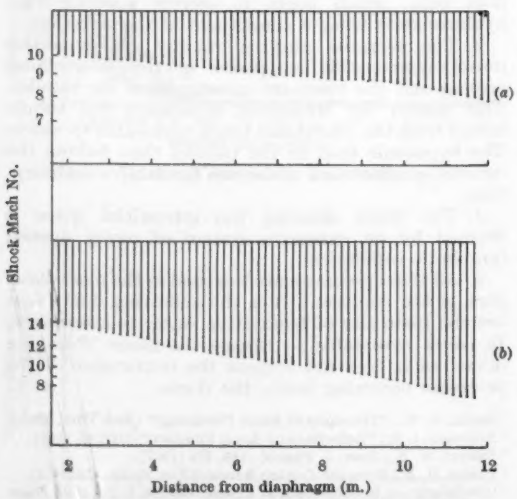

Fig. 1. Comparison of attenuation of shock waves in argon, initially at room temperature and 3·0 mm. pressure, using as driver: (*a*) hydrogen at room temperature and 32·7 atmospheres pressure; (*b*) a combustion mixture of hydrogen at 6·8 atmospheres pressure with 10 per cent oxygen

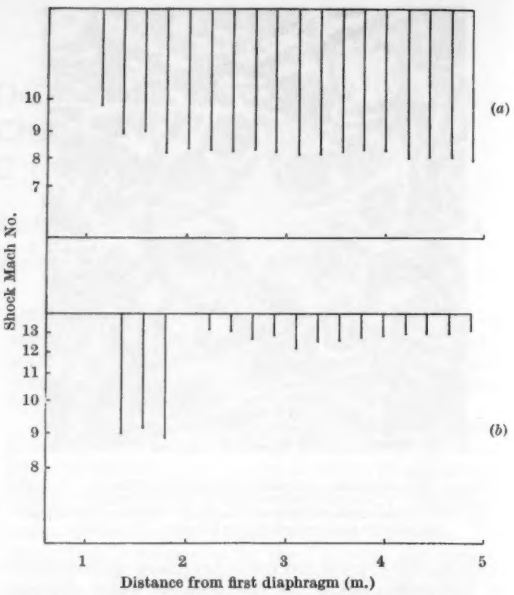

Fig. 2. Comparison of shock velocities using single and double diaphragms. The gases, initially at room temperature, and pressures as follow, are: (*a*) driver, hydrogen at 17·7 atmospheres; test section, argon at 5·0 mm.; (*b*) driver, hydrogen at 17·7 atmospheres; intermediate section, argon at 3·0 mm.; test section, air at 0·3 mm.

Attenuation is a most important consideration when a long shock tube is used to obtain a proportionately long column of flow at high Mach number. The attenuation is influenced not only by the diameter and surface construction of the walls of the tube, but also by lack of uniformity or turbulence in the action of the driver. Fig. 1 shows for comparison the shock velocities obtained in argon in a steel shock tube of internal section 5·4 cm. square using an aluminium diaphragm when the gas in the high-pressure section is (*a*) hydrogen at room temperature and (*b*) a combustion mixture of hydrogen with 10 per cent oxygen. With the cool hydrogen as driver, the shock velocity which initially corresponded to a Mach number of 10 (relative to the argon in its initial state at room temperature) fell to about Mach 8 in a distance of 10 m. When heated by combustion, the driver produced a higher initial velocity, equivalent to nearly Mach 14, but in a distance of 10 m. the Mach number had fallen to about 7. This greater attenuation is attributable to the imperfect state of the combustion mixture. Although it may be possible to improve the combustion technique, the serious attenuation shown in Fig. 1*b* occurred when a heated wire was held lengthwise down the high-pressure section to attain uniformity in the combustion process. Previous trials of multiple ignition points had proved far less satisfactory.

The experiments on the alternative method using an intermediate section were performed in a shock tube made of silver-plated brass having a circular section of internal diameter 5·1 cm. Typical results obtained in this tube with a single diaphragm of aluminium, again using hydrogen at room temperature as driver, are shown in Fig. 2*a*. Excluding the initial

stages during shock formation, the velocity of the shock wave decreased only slightly during its transit along the 5-m. distance from the diaphragm to the end of the tube; the attenuation is of the same order as that shown in Fig. 1*a*, which corresponds to about 0·2 in Mach number per metre. A second diaphragm was then introduced in order to form an intermediate section. The second diaphragm is relatively weak, as it is required only to withstand the comparatively low pressure in the intermediate section and to break when the intermediate shock wave impinges on it. A suitable material is 'Melinex', a polyester film manufactured by Imperial Chemical Industries Ltd., which also has the advantage of transparency to the 6-cm. radio waves used for the Doppler system of velocity measurement. The radio probe could thus look through the second diaphragm to observe the oncoming shock in the intermediate section as well as the shock produced in the final section. Fig. 2*b* shows the results obtained using hydrogen in the high-pressure section, and argon and air in the intermediate and final sections respectively, all the gases being initially at room temperature. With the pressure ratios noted on the diagram, a Mach number of 9 was obtained in the intermediate section jumping to Mach 12 in the final section and showing no evident attenuation. These results indicate that the double-diaphragm method appears to be much more promising than the combustion method for attaining high shock velocites in the shock tube without undue attenuation.

We are indebted to M. Woolley for his help in adapting the Doppler technique to the circular-section tube.

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Mode of Growth of Ice Crystals in Air and other Gases

It is widely known that the habit of ice crystals grown in air depends markedly on the temperature and excess of water vapour. This dependence was first studied by Nakaya and his collaborators and their results have been confirmed by many other investigators, but the problem of the factors determining habit remains unsolved.

The change of the ice crystal habit at water saturation vapour pressure with temperature is as follows: between 0° C. and about −3° C. ice crystals grow to thin hexagonal plates without internal design; between about −5° C. and −9° C. hexagonal prisms or columns are formed, between −10° C. and −20° C. hexagonal plates are again formed but thicker than at the higher temperature-range and with intricate design, with the frequent occurrence also of stellar forms and dendrites; and below −20° C. ice crystals grow along their principal axes in complex prismatic shapes. Marshall and Langleben[1] stated that the dependence of ice-crystal habit on temperature and supersaturation can be explained in terms of water-vapour excess over ice-saturation, whereas the experiments of Shaw and Mason[2] showed that the crystal habit is determined primarily by temperature, while the degree of supersaturation only modifies it. Kobayashi[3] came to the same conclusion as a result of experiments similar to those made by Nakaya *et al.* I have suggested[4] that the rate of diffusion of water vapour in air and the thermal conductivity of air play important parts in determining the mode of growth, and consequently that ice crystals should grow in different modes in air at a low pressure and in gases into which water vapour diffuses at different rates.

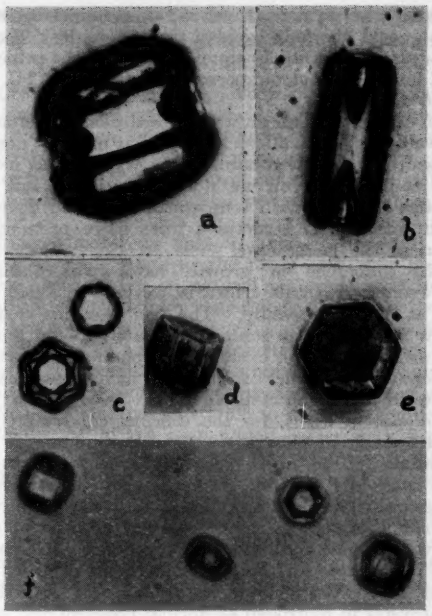

Fig. 1. Ice crystals grown in gases saturated with water vapour (×700). (*a*) Hydrogen at −7° C., (*b*) carbon dioxide at −7° C., (*c*) air at normal pressure at −16° C., (*d*) and (*e*) air at low pressure (40 mm. mercury) and at −16° C., (*f*) hydrogen at −16° C.

Experiments were therefore carried out on the growth of ice crystals in air at a low pressure and in various gases at temperatures of about −7° C. and −16° C. at water saturation; as is well known, between these temperatures a marked change in the growth habit of ice crystals is observed in air at normal pressure, since ice crystals grow preferentially along their principal axes in columnar form at −7° C. and along their lateral axes in plate-like or stellar forms at −16° C.; skeletal structures develop at both temperatures in air.

My experiments involved seeding a supercooled water cloud with silver iodide smoke in a cold chamber. In an atmosphere of hydrogen at normal pressure and in air at low pressures (80–20 mm. mercury) ice crystals grew in similar modes at −7° C. and at −16° C. which were very different from those in which they grew in air at normal pressure; in the former cases they grew to short and thick columns and skeletal structures did not develop in them. This tendency was more easily observed in air at low pressure than in hydrogen (Fig. 1). The diffusion coefficients of water vapour in hydrogen and in air at the low pressures employed are respectively 3·4 times and 10 or more times as large as that in air at normal pressure. Experi-

ments on ice crystals grown on rabbit hairs suspended in the cold chamber yielded similar results: the ice crystals grew at nearly equal rates along the principal and lateral axes at temperatures between −7° C. and −16° C.

In carbon dioxide (in which the diffusion coefficient of water vapour is 60 per cent of that in air at normal pressure) the growth habit of ice crystals was nearly the same as in air at normal pressure, but the change in habit with temperature and the development of skeletal structures were more pronounced than in the latter case.

No difference in growth habit was observed between ice crystals grown in oxygen, nitrogen and air at normal pressure. The fact that ice crystals grow in nearly the same mode in hydrogen and in air at low pressure, excludes the possibility that the difference in thermal conductivity at normal pressure between hydrogen and air might give rise to the difference in habit, for the thermal conductivity of hydrogen is much greater than that of air at low pressure (80–20 mm. mercury) which, in turn, is not markedly different from that of air at normal pressure. The results obtained with the gases mentioned above show that molecular adsorption of a particular gas, hydrogen or oxygen or nitrogen, is not responsible for the particular mode of growth in that gas. The characteristic mode of growth of ice-crystal in hydrogen at normal pressure and in air at low pressure may be considered to be due to the high rate of diffusion of water vapour through the gases.

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July 18.

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The High-Energy Proton Beam as a Neurosurgical Tool

Irradiation with high-energy heavy particles is suitable for the destruction of small selected regions in the central nervous system. Tobias *et al.*[1] irradiated the hypophysis of animals and man with 190-MeV. deuterons and 340-MeV. protons. Anderson *et al.*[2] made lesions in the hypothalamus of the rat using 190-MeV. deuterons. Malis *et al.*[3], making use of the thin ionization peak at the end of the beam, produced lesions at a depth of 0·8 mm. in the cerebral cortex of the cat with 10-MeV. protons. The continuation of animal experiments and the possible application of heavy-particle beams to stereotaxic radiosurgery in man[4,5] make it essential to know their effects on the central nervous system in detail.

A summary of work of the past two years is given, showing that with high-energy protons a sharply delimited lesion can be made at any desired site in the central nervous system. In the experiments some 50 rabbits and 5 goats were used. Only lesions observed up to 2 months after irradiation are considered here.

Irradiation was performed by a 185-MeV. proton beam from the 230-cm. synchrocyclotron. By focusing magnets and sweeping coils an almost parallel beam with a uniform cross-section of variable size was obtained. The desired beam profile was determined by a final aperture. The mean dose-rate was 1,000–2,000 rad per min. Dose distributions were calculated from density measurements on photographic films exposed in a water phantom and calibrated in terms of relative dose.

Transection of the spinal cord. Beams 1·5 and 10 mm. broad were directed across the spinal cord of rabbits. Lesions appeared following doses of 40,000 and 20,000 rad on the third and ninth day respectively. The lesion was sharply defined and in the white matter had the same width as the beam; it was slightly narrower in the grey matter. The changes after irradiation with the 1·5-mm. beam consisted of necrosis of nerve cells, axons, myelin sheaths and glial cells. Small perivenous hæmorrhages were present at the margin of the lesions and occasionally in the centre. A week after the onset of paresis, gitter cells and some glial cells containing iron were seen at the edge of the necrosis, and within 2–3 weeks large numbers of gitter cells were observed in sharply demarcated cystic spaces in the white matter. Slight proliferation of astrocytes occurred around the lesion. Using a 10-mm. beam the tissue reactions were more severe. There were multiple and sometimes rather large hæmorrhages as early as 3 days after irradiation. The results are reported by Larsson *et al.*[6].

Transverse lesion of the cerebral hemispheres. A beam 1·5 mm. broad was directed transversely through the upper part of the frontal lobes of rabbits. The dose was 20,000 rad. The rabbits remained in good condition and were functionally unaffected. The earliest lesions observed, 14 days after irradiation, and the changes between 2 and 8 weeks were similar. Corresponding to the path of the beam, a groove 2 mm. wide appeared on the upper surface of the hemispheres, and beneath it, in both the grey and white matter, a sharply defined narrow band of discoloration was seen. Within the lesion (Fig. 1) necrosis of nerve cells, myelin sheaths and axons occurred. Minute hæmorrhages were present in the damaged tissue, particularly in the grey matter. Collections of gitter cells were seen in the necrotic zone and around it proliferation of astrocytes.

Restricted lesion in the depth of the brain. Lesions were produced in the internal capsule of the goat

Fig. 1. Lesion of a rabbit's brain 2 weeks after irradiation with a proton beam 1·5 mm. broad. Dose, 20,000 rad. Small hæmorrhages occur in the damaged tissue, particularly in the grey matter. Azan. (× 8)

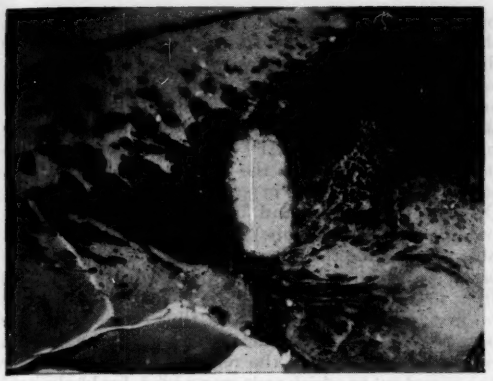

Fig. 2. Lesion in the internal capsule of a goat's brain 7 weeks after multiple port irradiation with a proton beam 2 mm. × 7 mm. Dose, 20,000 rad. Note the sharp demarcation of the lesion. Gros-Thionin. (× 4)

by the stereotaxic method[7], using multiple-port irradiation. A beam 2 mm. × 7 mm. or 2 mm. × 10 mm. and 20–22 fields were used, giving a dose of 20,000 rad at the desired site. The animals remained in good condition. Examined 4 and 7 weeks after irradiation, they presented restricted lesions in the selected areas (Fig. 2). The damaged region was lentiform and about 3 mm. × 7–10 mm. × 13–16 mm. The myelin sheaths, axons and glial cells within the lesion were completely destroyed. Slight cellular reaction was seen around some vessels at the periphery of the lesion. No hæmorrhages were seen in or around the necrotic zone, and there was no marked proliferation of astrocytes in the neighbourhood of the destroyed tissue.

With a narrow proton beam it is therefore possible to produce sharply delimited lesions of a desired site in any region of choice in the central nervous system. It must be stressed, however, that the observations apply only to lesions in their relatively early stages. The method is convenient for animal experiments.

To what extent such procedures may be used in operations on man depends on the late changes following high doses of proton radiation. Work in progress will demonstrate the long-term effects of such irradiation on the brain.

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A New Approach to the Investigation of the Cause of Moisture Expansion of Ceramic Bodies

Although moisture expansion of ceramic bodies has been studied by many investigators its cause is still not known with certainty. The hydratable materials that play a part may be anhydrous clay minerals[1,2], γ-alumina[3], amorphous silica (silica gel)[4,5], alumino-silicic acid gel[5] and permutites, zeolites or glass[1–3,6,7]. These are all amorphous (or poorly crystalline) materials that might be expected to appear at some stage in the firing of a clay body. In the past, studies on natural materials have been complicated by complex mineralogy and/or the presence of impurities, and it has been difficult to assess the influence of each component on moisture expansion. To overcome this we have studied moisture expansion using pure co-precipitated and mixed gels of silica and alumina and some to which an impurity of sodium oxide has been added. The work forms part of an investigation into the nature and cause of the permanent moisture expansion of ceramic bodies being carried out in this Division.

The moisture expansions produced by autoclaving fired test pieces of co-precipitated gels in saturated steam at 200° C. until expansion ceased are illustrated in Fig. 1. On firing to low temperatures pure alumina gel has a high moisture expansion due to the rehydration of γ-alumina to boehmite. At high temperatures of firing the alumina gel is transformed into α-alumina, which is moisture stable. Pure silica gel shows a moisture expansion at all temperatures of firing, but this drops as cristobalite forms. All moisture expansion curves show minima at the composition of mullite, but for low temperatures of firing the formation of mullite is not complete and an appreciable moisture expansion results from the presence of unreacted alumina and silica gels. Between the composition of mullite and pure silica gel the moisture expansion curves pass through maxima which are near the composition of kaolinite and which fall as the

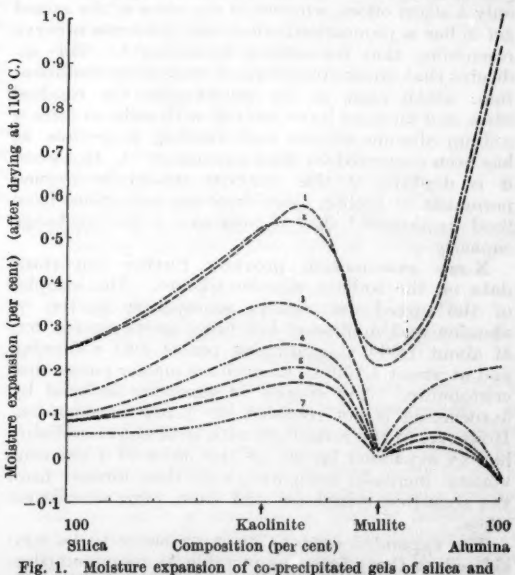

Fig. 1. Moisture expansion of co-precipitated gels of silica and alumina fired at temperatures: (1) 850°; (2) 900°; (3) 950°; (4) 1,000°; (5) 1,050°; (6) 1,100°; (7) 1,200° C.

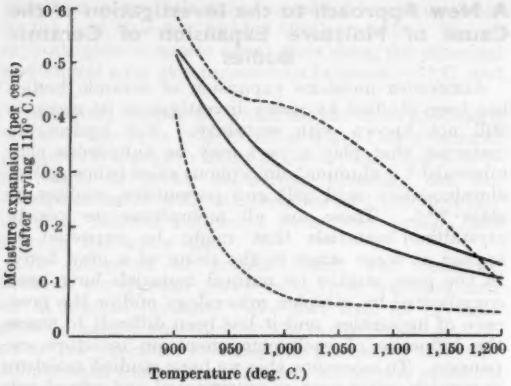

Fig. 2. Moisture expansion of pure and impure (addition of 2 per cent sodium oxide) gels with silica/alumina ratios of 2 formed by (1) co-precipitation and (2) mixing of the two components and fired at temperatures between 900–1,200° C. Top to bottom: mixed gel and sodium oxide; co-precipitated gel and sodium oxide; co-precipitated gel; mixed gel

firing temperature is increased. The only crystalline phase in this region is mullite, but there is much amorphous matter present which may be likened to Holdridge's alumino-silicic acid gel[5]. Between the composition of mullite and pure alumina gel the moisture expansion curves show a second series of maxima. These are considered to be due to the rehydration of γ-alumina to boehmite since the presence of silica prevents the formation of α-alumina even at a firing temperature of 1,200° C.

Particularly significant results are obtained by studying the moisture expansion after firing of pure and impure (addition of 2 per cent sodium oxide) gels with silica/alumina ratios of 2 (the composition of kaolinite) formed (1) by co-precipitation, (2) by mixing wet precipitated gels of the two components. The moisture expansion curves for the co-precipitated gels (Fig. 2) show that the addition of the alkali has only a slight effect, whereas in the cases of the mixed gel it has a pronounced effect and produces a curve resembling that for natural kaolinites[1,2]. This indicates that under conditions of incomplete mullitization, which exist in the mixed gels, the residual silica and alumina have reacted with soda to form a sodium alumino-silicate with swelling properties, as has been suggested for fired kaolinites[1–3,6]. However, it is doubtful if this material should be termed permutite or zeolite, since there are indications from fired kaolinites[2,3] that it possesses a low exchange capacity.

X-ray examination provides further important data on the sodium alumino-silicate. The samples of the mixed gel contain amorphous matter, γ-alumina and mullite at low firing temperatures, but at about 1,000° C. γ-alumina passes into α-alumina and at about 1,150° C. amorphous matter passes into cristobalite. Any change in structure induced by autoclaving is not revealed by X-ray examination. However, in the mixed gel with soda the cristobalite has an expanded lattice (of the order of 4 per cent volume increase) compared with that formed from the soda-free mixed gel and from pure amorphous silica.

The expanded cristobalite is probably to be considered as the β-form, metastable at room temperature, since it showed no inversion when heated. Cristobalite with similar properties has been found in opal[8] and Fuller's earth[9]. Buerger[10] suggested that the failure of the β-form to invert on cooling may be due to very small crystal size, whereas Flörke[9] considered it due to the existence of disordered forms arising from irregular stacking sequences of Si-O tetrahedral layers. We consider that it is due to the substitution of some Al^{3+} for Si^{4+} in the tetrahedral framework with the balance of charge maintained by Na^{+} held in cavities of the structure. On this hypothesis, and because the moisture expansion drops as cristobalite crystallizes, it is reasonable to conclude that the precursor to the cristobalite is an amorphous sodium alumino-silicate produced from amorphous silica, γ-alumina and sodium oxide on firing at about 1,000° C. It thus seems correct to ascribe to this amorphous compound the rise in moisture expansion in the region 1,000–1,150° C. for the mixed gel to which soda was added, for kaolinite[1,2] and, within limits, for kaolinite to which additions have been made[2,3].

Moisture expansion of low-burnt ceramic bodies can be due to the rehydration of anhydrous clay minerals[1] and that of high-burnt bodies to the rehydration of glass[7]. Our results demonstrate that amorphous alumino-silicates, amorphous silica and γ-alumina can also play important parts, depending upon the composition of the body and the firing temperature.

A fuller account of these results is to be published elsewhere.

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Effect of Temperature on the Structure of Set Gypsum Plaster

Variations within the normal range of temperature do not exert a very profound effect upon the rate of hydration of gypsum plaster[1,2]. For example, Fig. 1 shows the maximum rate of increase of temperature and the induction period (defined as the time required for the rate of increase of temperature to reach 0·1° C. per min.) plotted against initial temperature for a commercial plaster consisting predominantly of the so-called hemi-hydrate of calcium sulphate. The plaster had been allowed to age until it gave steady results. The reactions were carried out in a Dewar flask so that the increase in temperature is a valid parameter of the reaction. It is seen that the induction period is constant from 0° to 40° C., above which temperature it increases gradually. The maximum rate reaches a feeble maximum at about

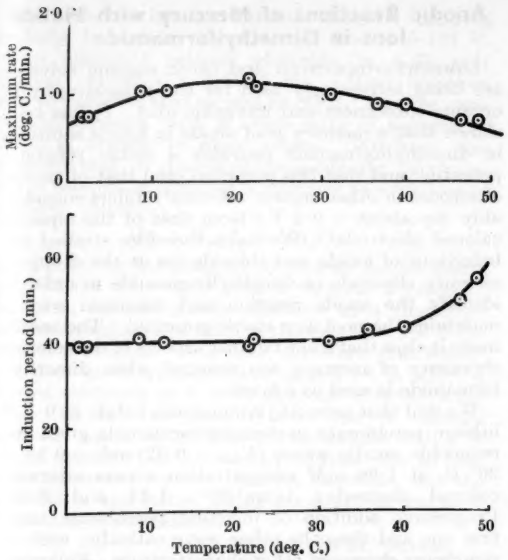
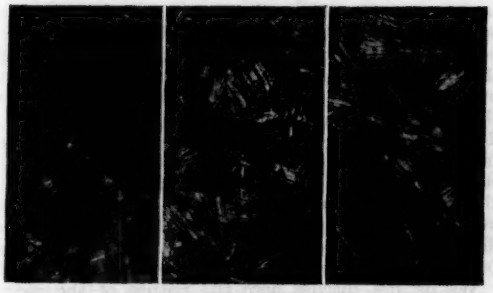


Fig. 1. The maximum rate of increase of temperature and the induction period plotted against initial temperature for a mixture of 100 gm. plaster in 60 ml. water

25° C. It appears, however, that at higher temperatures the rate of hydration does drop considerably[3]. Results similar to those shown in Fig. 1 were obtained for another plaster from a different source.

I have developed a method for examining the structure of set plaster under the microscope[4], and Fig. 2 shows the structures developed by a mixture of 100 gm. of plaster in 60 ml. water when setting took place at 0°, 20° and 46° C. The plaster belonged to the batch the kinetic characteristics of which are shown in Fig. 1, and it is seen that, despite the comparatively feeble effect on the rate of the setting process, temperature exerts a profound effect on the structure after setting has taken place. The differences are so great that the material produced at one temperature cannot be recognized from its appearance when prepared at another temperature. At 46° C. a sheaf-like habit predominates, whereas at 20° C. swallow-tail twins and an acicular habit are the rule. At 0° C., a fine structure which could not be fully resolved was obtained. Examination by X-rays showed that in no case was any material other than well-crystallized gypsum present in detectable quantities. The grain sizes of the specimens cast at the three different temperatures (Fig. 2) show that not only does the habit of the gypsum change as the temperature is raised but also that the number of effective nuclei decreases. It is also evident that the average pore size becomes much larger. Similar results were obtained for six commercial plasters representing gypsum deposits in four States of the Commonwealth of Australia.



(a) (b) (c)

Fig. 2. The structure developed in a mixture of 100 gm. of plaster in 60 ml. water set at various temperatures: (a) 0°; (b) 20°; (c) 46° C.

The extreme members of the series shown in Fig. 2 refer to conditions outside the range of experience in the plaster industry. However, further work has revealed that the type of structure shown in Fig. 2*a* may extend to about 10° C. or even higher, and that the high-temperature type is dominant at 30° C. In fact, a considerable variation of structure is possible over the range of practical working temperatures.

It is expected that changes in structure will be associated with changes in other properties. Preliminary experiments confirm that the breaking strength is appreciably affected. For example, specimens cast at 0° C. are about 12 per cent stronger[1,5] than those cast at 29° C. Work carried out in this Division indicates that structural changes of this type may be associated with changes in the adhesion of paint films (Hoffmann and Ridge, unpublished results) and in the expansion on setting.

It is suggested that the properties of plaster after setting may be more dependent upon temperature than was previously thought, and that some of the sporadic problems of the plaster industries, such as the 'crazing' of fibrous plaster sheets, may be associated with unusual conditions of temperature at the time of casting.

This investigation forms part of the programme of work carried out within the Division of Building Research and sponsored by the Associated Fibrous Plaster Manufacturers of Australia.

Thanks are due to Dr. W. F. Cole for the X-ray diffraction patterns.

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A New Method for obtaining Thin Plane Parallel Sections of Mineralized Specimens

Nowadays a number of micro-methods (for example, X-ray and interference microscopy, and isotope techniques) are used for quantitative analyses of mineralized biological tissues. These methods have a resolution which allows analyses at or below the cellular scale. Difficulties in preparing the biological material have so far been a limiting factor in the application of the methods. An ideal specimen is a thin, plane parallel tissue section with a known thickness within the region 10–100μ. In order to prepare such sections, blocks are generally ground

down to a desirable thickness, but all such methods so far used possess serious disadvantages: they do not allow a continuous control of the thickness during the grinding procedure, and wedge-shaped sections or fragments of sections are often obtained. These difficulties prompted us to seek a method for preparing ground sections based on lapping technique.

Mineralized tissue embedded in methyl methacrylate is used. The specimens are sawn into sections 3–4 mm. thick. One side of the section is lapped against cast-iron using very fine diamond abrasive, which gives the surface an extremely good quality with regard to both planarity and surface finish.

For the subsequent steps of the lapping procedure a small steel cylinder is used as a fixture for the specimen. A square-ruled area is etched on one of the plane surfaces of the cylinder, which are perpendicular to its axis. The etched lines are a few microns deep and have a width of about 50μ. On top of the etched area the lapped surface of the specimen is attached with the aid of a glue which is easy to work out to a thin film less than 0·5μ thick. When the glue is dry the specimen is cooled and ground by hand against an ordinary emery plate, down to about 1 mm. thickness.

The next step comprises grinding against a fine-grained diamond plate. The fixture is moved by hand in a V-shaped prism which is oriented perpendicularly to the rotating plane of the grinding plate. At this stage, a section of about 100μ is obtained within a few minutes. The shape of the fixture allows continuous approximate measurements of the specimen with the aid of an ordinary micrometer.

In the last step the specimen is lapped against a cast-iron plate using fine-grained diamond abrasive, the fixture being oriented with its longitudinal axis perpendicular to the lapping plane. The sections are lapped by hand to the desired thickness, checked by repeated measurements. A coarse control of the thickness of about $\pm$ 2μ can be obtained with the aid of a good micrometer. A more accurate control of thickness can be achieved with the aid of a 'microkator' (a Swedish mechanical measuring instrument, manufactured by C. E. Johansson). A complete topographical thickness-measurement, with the same lateral resolution as the light microscope can be obtained with the aid of a mechanical-optical instrument designed by Hallén[1].

Good sections have been obtained with our prototype equipment. For example, thickness measurements of a cross-section 17μ thick of the crown of a tooth was made. The thickness was measured at 100 arbitrarily distributed points within the dentin as well as the enamel. The mean thickness was 16·7μ, standard error 1·7, which gives a coefficient of variation of 10·3 per cent. The section is slightly wedge-shaped with a slope of 1/5,000.

A more detailed report will be published elsewhere.

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Anodic Reactions of Mercury with Halide Ions in Dimethylformamide

Dimethylformamide and other organic solvents are being increasingly used for the polarography of organic substances and inorganic ions. It has been shown that a mercury pool anode in halide solutions in dimethylformamide provides a stable reference potential[1] and that this potential (and that of similar electrodes in other organic solvents[2]) differs considerably (by about − 0·5 V.) from that of the aqueous calomel electrode[1]. We have therefore studied the behaviour of iodide and chloride ion at the dropping mercury electrode in dimethylformamide in order to identify the anode reaction and ascertain why it maintains the pool at a stable potential. The results make it clear that some familiar aspects of the solution chemistry of mercury are changed when dimethylformamide is used as solvent.

We find that tetraethylammonium iodide in 0·1 *M* lithium perchlorate in dimethylformamide gives two reversible anodic waves ($E_{1/2}$ − 0·32 and + 0·10 at 20° C. at 1·96 m*M* concentration versus saturated calomel electrode; $i_d/cm^{2/3}t^{1/6}$, 1·24 and 0·50). Progressive addition of mercuric perchlorate made first one and then the other wave cathodic, with no significant change of other characteristics. Following the methods used by Kolthoff and Miller[3] in their study of mercury complexes in water we assumed the electrode reaction of the more negative wave to be:

$$Hg + aX' \rightleftharpoons HgX'^{(a-2)-} + 2\varepsilon \qquad (1)$$

for which the potential of the dropping electrode would be given by:

$$E_{de} = \text{const.} + \frac{RT}{2F} \ln \frac{i}{(i_d - i)^a} \qquad (2)$$

We plotted E_{de} for the more negative wave against the logarithmic function of equation (2) for various values of the exponent a. If one takes $a = 4$ a good straight line of slope 0·029 ($= 2{\cdot}303RT/2F$) results; the electrode reaction is therefore:

$$Hg + 4I' \rightleftharpoons HgI_4'' + 2\varepsilon \qquad (3)$$

When 0·1 *N* tetraethylammonium iodide is used as the supporting electrolyte in polarography in dimethylformamide the galvanometer zero is at − 0·55 V. versus standard calomel electrode; hence one is limited to the study of substances of half-wave potential more negative than this, since at less-negative potentials the mercury micro-electrode dissolves, according to equation (3). Similarly, when a diffusion current passes through the cell owing to reduction of a substance in the solution, the same reaction must occur on the mercury pool if this is used as anode. We suggest that the stability of the pool potential is to be accounted for by supposing that complex formation maintains a concentration of mercury ions in solution sufficiently high to ensure that further quantities, produced by electrolysis, do not increase the total concentration by a significant fraction. It should be borne in mind that the overall stability constant of the complex ion is high[4], and that there is a large and constant excess of iodide ions (the stability constants of the halide complexes in dimethylformamide, which we have estimated from polarographic data, will be reported elsewhere).

We do not discuss here the nature of the more positive wave given by iodide ion, because it is not relevant to the behaviour of the anode in polarography in iodide as supporting electrolyte.

The chloride ion (as lithium chloride) behaves similarly to iodide ($E_{1/2}$, $-0{\cdot}17$ and $+0{\cdot}125$ V. versus standard calomel electrode in 4·97 m*M* solution; $i_d/cm^{2/3}t^{1/6}$, 1·16, 0·53). Here, however, there is evidence that as well as $HgCl_4''$ another complex of mercury, with a lower ratio of halogen to metal, is formed to some extent. We have also observed that in dimethylformamide the precipitation of calomel on addition of mercury to a solution of mercuric chloride:

$$Hg + HgCl_2 = Hg_2Cl_2$$

is prevented by the presence of chloride ion (in water, precipitation does occur even if chloride is present). Moreover, calomel slowly dissolves in a dimethylformamide solution of lithium chloride (but not if the chloride is absent). It is clear, therefore, that a mercury pool in chloride solution cannot be in effect a calomel electrode as it is in aqueous chloride solutions. In fact we have found[1] that a 'calomel electrode' made up in dimethylformamide with 2 *N* lithium chloride has initially a potential close to that of the saturated aqueous calomel electrode, but the potential is not stable, owing no doubt to the slow dissolution of the calomel.

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A Theoretical Expression for Solubility Product

In view of the great importance of the 'solubility product' in chemistry, it was considered desirable to obtain a theoretical expression, to explain the observations described in a previous communication[1] and also to correlate the solubility product with some significant properties of the ions which combine to form the precipitates.

When two dilute aqueous solutions of electrolytes (for example, barium chloride and potassium sulphate) are mixed, the oppositely charged ions (Ba^{2+} and SO_4^{2-}) are brought into close association owing to electrical attraction and combine to form molecules of the precipitate (barium sulphate). The attraction is caused partly by Coulomb forces and partly by the mutual polarization of ions (ion–dipole interactions). The potential energy of a system consisting of oppositely charged ions is thus:

$$U = \left\{ -\frac{(z_+\varepsilon).(z_-\varepsilon)}{R_0}\left(1 - \frac{1}{n}\right) - \frac{\alpha_a(z_+\varepsilon)^2 + \alpha_c(z_-\varepsilon)^2}{2R_0^4}\right\} \quad (1)$$

where z_+ and z_- are valencies of the cation and anion, ε is the electronic charge, α_c and α_a are polarizabilities of the positive and negative ions, R_0 the equilibrium distance between the centres of the two ions, and n, a constant (about 9) occurring in Born's repulsion-energy term.

Redispersion of these ions from the precipitate does not take place unless they possess energies exceeding E (equal to $-U$). The stronger the binding forces between the ions, the smaller is their tendency to pass out into solution as free ions, and hence the greater the insolubility of the precipitate formed.

The velocity of recombination of the oppositely charged ions in solution, which is an instantaneous process (for which the energy of activation is practically zero), is:

$$k_1.(n_{Ba^{2+}}\ n_{SO_4^{2-}}) \quad (2)$$

where $n_{Ba^{2+}}$ and $n_{SO_4^{2-}}$ are the numbers of these ions per unit volume (concentrations) and k_1 is a constant.

The velocity of the reverse process, namely, the redispersal of ions from the surface of the precipitate into solution, is:

$$k_2.A.n_0 \exp(-E/RT) \quad (3)$$

where A is area of the interface of the particles of the precipitate and n_0 is the number of pairs of ions (Ba^{2+} and SO_4^{2-}) per unit area of the surface. At equilibrium:

$$k_1.(n_{Ba^{2+}}\ n_{SO_4^{2-}}) = k_2.A.n_0.\exp(-E/RT) \quad (4)$$

But by definition $(n_{Ba^{2+}}\ n_{SO_4^{2-}}) = S_P$, the solubility product of barium sulphate. Introducing the value of E given in (1) we have:

$$S_P.\left(\frac{k_1}{k_2An_0}\right) = \exp(-E/RT) = \exp\left[-\left\{\frac{z_+z_-\varepsilon^2}{R_0}\left(I - \frac{}{n}\right) + \frac{\alpha_a z_+^2\varepsilon^2 + \alpha_c z_-^2\varepsilon^2}{2R_0^4}\right\}\Big/RT\right] \quad (5)$$

S_P is a constant at constant temperature if A is constant. Thus,

$$-\log_e S_P = \frac{E}{RT} + a = \left\{\frac{\alpha_a z_+^2\varepsilon^2 + \alpha_c z_-^2\varepsilon^2}{2R_0^4}\right\}\Big/RT + a \quad (6)$$

since for very small values of R_0 the first term in (1), namely $\left(\frac{z_+z_-\varepsilon^2}{R_0}\right)$, can be neglected in comparison with the second term representing the mutual ion-dipole interactions. If temperature is constant, expression (6) reduces to:

$$-\log_{10} S_P = K(\alpha_a z_+^2 + \alpha_c z_-^2) + a \quad (7)$$

(K_1 and a are constants. For compounds of similar chemical constitution R_0 is nearly constant.) We should therefore expect to obtain a straight line by plotting $-\log_{10}S_P$ against $(\alpha_a z_+^2 + \alpha_c z_-^2)$. This is in agreement with results represented graphically in Fig. 1, obtained from known values of S_P and the polarizabilities of the constituent ions.

It is known that $\alpha = r^3$ for a spherical molecule, assumed to be a perfect conductor. Applying this to ions, we have:

$$-\log_{10} S_P = K_1\left\{\frac{r_a^3 z_+^2 + r_c^3 z_-^2}{2R_0^4}\right\}\Big/RT + a \quad (8)$$

If we now compare salts which are chemically similar, having one common ion and the other ion having the same valency (T being constant), equation (7) becomes:

$$-\log_{10} S_P = K_1'\alpha + a = K_1' r^3 + a' \quad (9)$$

This explains our previous observation[1] that the most insoluble inorganic precipitates are formed from hydrated ions having the largest size (including the hydration layer). On plotting $-\log_{10}S_P$ against $1/d^3$, where d is the distance of the diffusion front of each band from the starting point of diffusion, when we examine the bands in a chromatogram produced in an agar-gel layer containing the appropriate reagents during the diffusion of the electrolytes, then

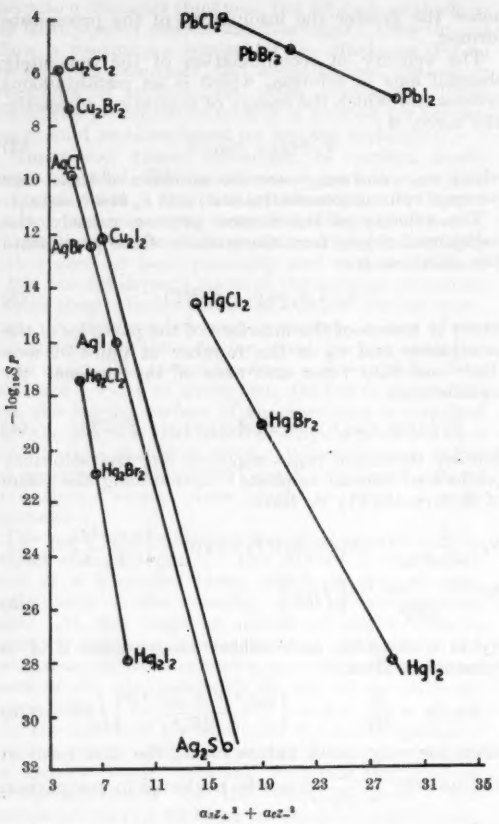

Fig. 1

straight line graphs are obtained, confirming the correctness of equation (9).

Equation (9) is consistent with the view that ions having larger electrical fields of force due to the combined effect of the electrical charge of the ions and their polarizability can induce dipoles in the water molecules which surround them, and produce correspondingly larger ion-dipole attractions between the ions and water molecules. It follows that when this ion-dipole interaction is stronger, the hydration of the ions is greater. This explains how the formation of the most insoluble precipitates is brought about by the combination of the most hydrated ions, leading to the release of the largest amount of water of hydration and consequently the largest increase in volume[1].

Further, it has been found that the polarizability (α) is directly proportional to N^2 (N being the atomic number of the element constituting the ion) in the case of the ions examined by me, for which the values of α are available. On substituting this relation, equation (9) can be rewritten as:

$$-\log_{10} S_P = K_1'(N^2) + a' \qquad (10)$$

This is completely borne out by actual calculations based upon the known values of the solubility products and atomic numbers of the constituent ions.

Equation (6) represents correctly the variation of the solubility product with temperature. From the assumptions made in the derivation of equation (4) it is clear that the solubility product should increase as the size of the particles of the precipitate becomes smaller, a fact observed by previous workers.

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Application of the Hanging Mercury Drop Electrode to an Investigation of Intermetallic Compounds in Mercury

IN the application of the hanging mercury drop electrode to analytical determination of trace metals we have used very dilute solutions[1] in which no mutual interaction of metals was observed, even in the presence of more than one metallic species. We wish to report an investigation of the conditions in which such interaction effect cannot be neglected.

In a preliminary investigation of more concentrated complex amalgams we have observed that the anodic oxidation currents of individual metals may decrease or even disappear completely. This can sometimes be connected with a change in the anodic potentials for dissolution, which suggests that the formation of intermetallic compounds with mercury must occur. Therefore, in the analytical method of concentrating metals in mercury previously described[1] this must be taken into account when working with high concentrations (greater than 10^{-5} M). Application of this method to the study of the formation of intermetallic compounds can be illustrated with reference to the nickel–zinc system.

In Fig. 1 curves of dissolution of amalgams formed at the hanging mercury drop electrode are given. The electrolysis time was 2 min. The electrolysis was performed at the potential $-1{\cdot}4$ V. versus the saturated calomel electrode. $0{\cdot}1\,N$ potassium chloride was used as a supporting electrolyte to which various amounts of nickel and zinc sulphates were added. The current – voltage curves were recorded polarographically in the range $-1{\cdot}4$ to $+2{\cdot}0$ V. In Fig. 1 the short horizontal lines indicate zero deflexion of the galvanometer, the effective sensitivity of which was 6×10^{-6} amp./mm./m. Curve 1 was recorded for pure supporting electrolyte. The increase of anodic current at a potential of about $+0{\cdot}1$ V. corresponds to dissolution of mercury. This soon stops, because formation of a calomel film on the mercury surface inhibits further dissolution of mercury and causes the current to decrease nearly to its initial value. Curve 2 was recorded after the addition of $5 \times 10^{-4}\,N$ zinc sulphate; the anodic dissolution of zinc is indicated by a minimum, close to $-1{\cdot}0$ V. The corresponding curve 6 was obtained with nickel present in the solution at

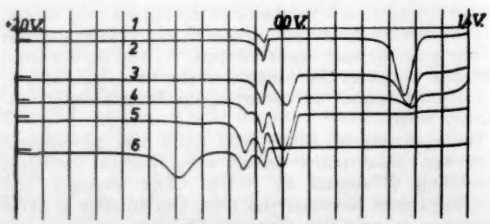

Fig. 1

a concentration of $2 \times 10^{-4} N$, anodic dissolution occurring at a potential of +0·25 V. Another flat minimum can be observed when the electrode is further polarized, near +0·8 V., which probably corresponds to oxidation of Ni^{2+} to Ni^{3+}.

Curves 3, 4 and 5 were obtained for constant concentration of zinc ions ($5 \times 10^{-4} N$) and different concentrations of nickel ions, with all other experimental conditions constant. For a nickel concentration of $2 \times 10^{-4} N$ (curve 3) a nickel oxidation minimum does not occur, in spite of the fact that for the same concentration of nickel in the absence of zinc this can be readily observed. In this experiment the zinc minimum is also diminished. A new anodic minimum was noted at −0·1 V. Those changes are more pronounced (curve 4) when the nickel concentration is further increased ($4 \times 10^{-4} N$). When it reaches $6 \times 10^{-4} N$ the dissolution current for zinc almost entirely disappeared and the currents corresponding to nickel dissolution, and also its oxidation, can be observed as in curve 6.

These observations can be explained as follows. Zinc and nickel in the amalgam form an intermetallic compound with a certain composition. When there is less than the stoichiometric quantity of nickel present than is required for the full formation of this compound, a decrease in the minimum for zinc is observed (curve 3) together with a new minimum corresponding to oxidation of the intermetallic compound. When excess of nickel is present, no zinc minimum can be found at all, but the nickel minimum appears, besides the new minimum due to the intermetallic compound (curve 5).

The possibility of reproducible polarization of the hanging mercury drop electrode covered with a calomel film is especially interesting. This film does not interfere in some electrode reactions, as was shown in connexion with nickel oxidation.

This method was also successful when other intermetallic systems were investigated, including Cu—Zn, Au—Zn, Au—Cd, Sn—Ni, Cr—Ni, Mn—Ni, Ag—Zn, Ag—Cd, Co—Ni, Mn—Fe.

A more detailed account of this work is in preparation.

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An Unusual Form of Corrosion Product

DURING an investigation into the influence of speed of flow on the internal corrosion of mild-steel tubes, an interesting and unusual form of corrosion product occurred. The tests were carried out with annealed 5/8-in. bore pipes through which Metropolitan Water Board water was flowing at 56° C. at a speed of 430 ft./min. The initial alkalinity of the water was 195 but fell rapidly during the first three days of the test to 75 and gave an overall mean of 76 (expressed as p.p.m. calcium carbonate). The mean *p*H value was 8·8.

Examination of sample lengths which had been on test for 4–10 weeks showed the formation of a rough very adherent scale on the surface with a number of 'whiskers' growing perpendicular to the tube surface

Fig. 1. Cross-section of whisker

for about 0·5 mm. and then curving and growing parallel to the tube surface and along the direction of flow. The length of these whiskers was up to 90 mm., the weight per cm. was 0·44 mgm., and the diameter varied from about 0·4 mm. at the point of attachment to less than 0·2 mm. at the growing tip. The rate of growth is appreciable, and can amount to more than 1 mm. per day. Examination of these whiskers under the microscope showed them to be hollow (Fig. 1). X-ray analysis of various sections showed them to consist of magnetite (Fe_3O_4) or goethite (αFeOOH) or a mixture of the two. A composite diagram based on measurements of a large number of whiskers is shown in Fig 2.

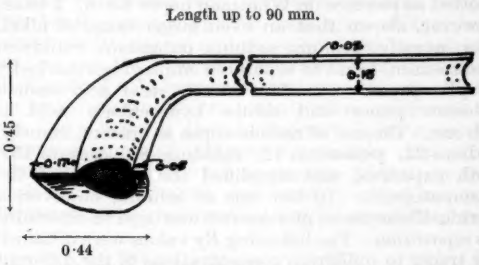

Fig. 2. Longitudinal section of whisker. Dimensions in millimetres

The following tentative mechanism for the formation of these whiskers is suggested. During the early stages of corrosion an adherent carbonate scale is thrown down by the hard water, but this is not sufficiently complete or repairable, since the alkalinity rapidly falls, to stifle corrosion completely, and corrosion takes place at local anodic points. In stagnant solution localized corrosion commonly leads to the formation of a hemispherical dome of corrosion product over a pit. It appears that under the influence of rapidly flowing water the symmetry of the form of corrosion product is disturbed, leading to the formation of a hollow cylinder bent through a right angle in the direction of flow. Ferrous ions, formed at the anodic area at the root of the whisker, diffuse along the tube, and growth of the whisker proceeds at the tip, where these ions are oxidized and precipitated as iron oxide on coming into contact with the aerated water. The rate of ionic diffusion

may be increased by the suction induced by the rapidly flowing water at the tip.

Mayne[1] has shown that rapid oxidation of ferrous hydroxide at a *p*H value greater than 5 gives goethite while slow oxidation leads to the formation of an intermediate between magnetite and ferric oxide (γFe_2O_3). Thus the formation of goethite in the present case is readily explained. The formation of magnetite, the second constituent, is not likely to occur in the same area at the same time as goethite since slow oxidation would appear to be necessary. Therefore, the oxidation to form this compound must occur in some areas sheltered from the direct action of the water. This may occur in pockets of ferrous ion-containing water trapped in the goethite crystals or by slow oxidation of the ferrous ion during its passage through the whisker either by a small amount of oxygen contained in the water or by oxygen diffusing through the wall of the tubule.

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Radiochromatography of Alkali Ions

In their recent communication[1], Modreanu, Fisel and Carpov state "only three mixtures of solvents seem to have been proposed to accomplish the chromatographic separation of potassium, rubidium and cæsium". These methods being "tedious", they use nitrobenzene saturated with water as solvent for the separation of potassium, rubidium and cæsium spotted as picrates on Whatman paper No. 4. I have, however, shown that an even larger range of alkali ions, namely, lithium, sodium, potassium, rubidium and cæsium, could be separated employing a markedly simpler procedure[2]. This merely used acid-washed asbestos paper and dilute hydrochloric acid as solvent. The use of radioisotopes as tracers, namely, sodium-22, potassium-42, rubidium-86, cæsium-137, both expedited and simplified the analysis of the chromatogram. In the case of lithium, however, a Perkin-Elmer flame photometer was used to determine its *répartition*. The following R_F values were obtained for tracer to milligram concentrations of the different alkali ions with 0·1 *N* hydrochloric acid as solvent on bands of asbestos paper 12 mm. wide, 25 cm. long and 0·2 mm. thick.

Li^+	Na^+	K^+	Rb^+	Cs^+
0·99	0·86	0·77	0·72	0·45

Results on electromigration of ions in fused salts[3] showed that heavier ions tended to become 'fixed' preferentially on the asbestos paper used as support. This was confirmed by their chromatographic separation with the same solvent (dilute hydrochloric acid) on Whatman filter paper treated with 'Dowex'-50 following Lederer's technique[4], as also on similar ion-exchange resin-coated cellulose filter paper supplied by Carl Schleisser and Schül[5]. From the similarity of results it was shown that asbestos functions as an ion-exchanger of feeble capacity.

In the study of the isotope effect in the electromigration of a mixture of cæsium-131 and cæsium-137 ions in fused sodium nitrate[5] this technique of asbestos paper chromatography was used to obtain cæsium free from sodium from samples initially containing more than a 1,000-fold excess of the latter. Simple ascending chromatographic development with distilled water as solvent sufficed for the complete separation of cæsium and sodium, the R_F values under these conditions being: Cs^+, 0·0, and Na^+, 0·75.

Grateful thanks are due to [the late] Prof. F. Joliot-Curie, in whose Laboratoire de Physique et Chimie Nucléaires, Collège de France, Paris, this work was done, and to Dr. M. Chemla for valuable guidance.

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A New Form of Tricalcium Phosphate

We have established that the α-form of tricalcium phosphate is not the highest-temperature form of this compound as hitherto believed[1–3]. At 1,430° C., the α-form inverts to the true highest-temperature polymorph, which is capable of forming an uninterrupted range of solid solutions with the α-form of dicalcium silicate. This new polymorph, which we have designated 'super-α-tricalcium phosphate', does not survive quenching to room temperature. Characteristic optical changes accompanying the α ⇌ super-α-tricalcium phosphate transformation were first observed by a high-temperature microscope technique which has been described[4], and the existence of the super-α-form was confirmed by high-temperature X-ray analysis using recently developed equipment[5].

Full details of this work will be published elsewhere.

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Structure of Cupric Mono-, Di- and Tri-bromoacetates

It was concluded in our former communication[1] that cupric mono- and di-chloroacetates in the crystalline state and in ordinary organic solvents might consist of the binuclear molecules with a sort of copper-to-copper linkage, though cupric trichloroacetate does not have a similar binuclear molecule in the crystalline state or in dioxan. The exceptional structure of cupric trichloroacetate may be ascribed to the accumulation of the inductive effect of the three chlorine atoms. In this connexion, it seems interesting to see what type of structure may be involved in the cupric bromoacetates. Therefore, we examined the structure of cupric mono-, di- and tri-bromoacetates, using the criteria previously derived: (1) the cupric monocarboxylates involving the binuclear molecules show, in addition to a band at about 40 to 44 × 10^{13} c./s., a special absorption band at about 80 × 10^{13} c./s., which was regarded as possibly

due to the presence of the copper-to-copper linkage; (2) the polarization of the latter band is the reverse of the polarization of the former.

The present measurements indicate that cupric mono-, di- and tri-bromoacetates in dioxan have an absorption band at about 80×10^{13} c./s. besides an absorption band at about 40×10^{13} c./s., suggesting that all these compounds may contain the acetate-type binuclear molecules. The dichroism measurements, which have been carried out in the visible and ultra-violet region by Tsuchida and Kobayashi's microscopic method[2], indicate that cupric monobromoacetate monohydrate and anhydrate show absorption bands at about 40 and 80×10^{13} c./s. in addition to absorption bands in the far ultra-violet. Moreover, the polarization of the latter band is bound to be the reverse of the polarization of the former. These relationships bear a close resemblance to those with the cupric alkanoates, suggesting the existence of the binuclear molecules in organic solvents and in the crystalline state.

Similar characteristics have been observed in the absorption spectra of cupric di- and tri-bromoacetates. Thus the two compounds, anhydrous and hydrated, show absorption at about 40 and 80×10^{13} c./s., besides strong absorption in the shorter wavelength region. In addition, the polarization of the latter band is the reverse of the polarization of the former. It is certain that cupric di- and tri-bromoacetates consist of the binuclear molecules in the crystalline state and in organic solvents.

The difference between the tribromo- and trichloroacetates, as observed in the present work, may be regarded as due largely to the greater inductive effect of the bromine as compared with that of the chlorine.

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Paper Chromatography of Small Amounts of Vasopressins and Oxytocins

IN work concerned with the composition and estimation of neurohypophysial hormones in the posterior pituitary, the hypothalamus and body fluids, it would be desirable to have a chromatographic procedure which permits the identification in and recovery of small amounts of the active peptides from impure extracts. It would also be useful to know whether the substituted synthetic oxytocins which have recently been prepared[1] can be distinguished by a simple chromatographic method. Taylor[2] separated oxytocin, and arginine- and lysine- vasopressin by ion-exchange chromatography on resin columns. He used du Vigneaud's highly purified peptides, and the smallest amounts applied were equivalent to 0·6 mgm. of this material. Benfey[3] purified Stehle and Fraser's[4] preparations of ox posterior pituitary hormones (200 U./mgm. pressor activity and 250 U./mgm. oxytocic activity) by paper chromatography using ninhydrin as the colour reagent. The smallest amounts of the hormone preparations used were 1 mgm. However, since only terminal amino-groups react with ninhydrin, it is obviously disadvantageous to use this reagent when attempting to visualize very small quantities of the posterior pituitary peptides. Rydon and Smith[5] have shown that micrograms of peptides of all kinds, including cyclopeptides, can be detected by chlorination followed by spraying with a starch–potassium iodide solution. Reindel and Hoppe[6] improved this procedure in two ways. First, before exposure to chlorine, they moistened the paper with a mixture of watery ethanol and acetone, which prevents background staining. Secondly, they treated the N-chloropeptides with a mixture of potassium iodide and either benzidine or *o*-tolidine in acetic acid and thereby increased the sensitivity of the method.

We used Reindel and Hoppe's modification for the ascending chromatography of synthetic oxytocin, valine-oxytocin, phenylalanine-oxytocin, arginine-vasopressin and lysine-vasopressin. Other preparations used were 'Pitressin', 'Pitocin' and 'Pituitrin' (Parke, Davis and Co.) and simple acetic acid extracts of ox, hog and rat posterior pituitary glands and hypothalami. The paper employed was Whatman No. 1, the solvent was *n*-butanol/acetic acid/water (5 : 1 : 4), the length of flow was 24–48 hr. and the experiments were done at ambient temperatures. Owing to the small amounts of peptides used (see below) some of the spots faded rapidly. All chromatograms were therefore photographed immediately after development.

Areas on parallel strips, corresponding to spots obtained from reference preparations, were extracted with Ringer–Locke solution, and the activities in the eluates estimated by bio-assay. The isolated rat uterus was used for oxytocic assays; pressor activity was assayed on the blood pressure of rats anæsthetized with urethane and treated with dibenyline.

Fig. 1*a* shows a chromatogram of highly purified arginine-vasopressin and lysine-vasopressin, which were kindly supplied by Prof. van Dyke. (Saline containing chlorobutanol and acetic acid was the solvent used by van Dyke for the vasopressins obtained from du Vigneaud for pharmacological experiments. Since the sealed ampoules were heated before refrigeration, van Dyke believes that biologically inert spots in chromatograms of arginine-vasopressin represent degradation products not necessarily present in the original material, which was highly purified but not the purest which du Vigneaud and his associates have

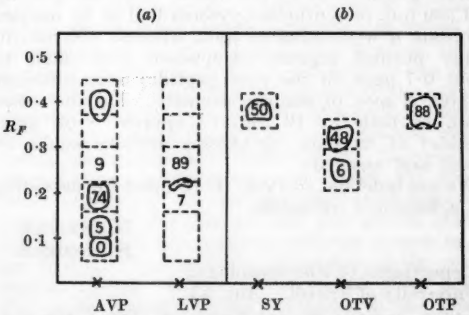

Fig. 1. Paper chromatograms of (*a*): arginine-vasopressin (AVP) and lysine-vasopressin (LVP), and (*b*): synthetic oxytocin (SY) ('Syntocinon', Sandoz), valine-oxytocin ('OTV 80', Sandoz) and phenylalanine-oxytocin ('OTP 79', Sandoz). Two strips of paper were chromatographed for each substance but only one was developed; the other was cut into pieces indicated by the broken lines. The pieces were extracted and the eluted activities were bioassayed. The figures within the columns indicate percentage recovery. The amounts (in mU.) applied at the origin *X* were: AVP, 800; LVP, 740; SY, 2,450; OTV, 300; OTP, 560

Table 1. R_F Values in *n*-Butanol/Acetic Acid of Vasopressins and Oxytocins

Peptide	R_F
Arginine-vasopressin	0·19
Lysine-vasopressin	0·26
Oxytocin (synthetic)	0·37–0·40
Valine-oxytocin	0·33
Phenylalanine-oxytocin	0·37–0·39

isolated. The latter have previously commented on the instability of arginine-vasopressin.) Arginine-vasopressin gave more than one spot but the bulk of the pressor activity was found at R_F 0·19. Most of the pressor activity of ox-gland extracts was found at approximately the same R_F. In the case of lysine-vasopressin 89 per cent of the activity applied was found above the clearly stained area at R_F 0·26. Experiments with pig posterior pituitary extracts, in which larger amounts of the hormone could be applied, yielded a faint spot at the same region, which contained likewise most of the pressor activity. Valine-oxytocin (Fig. 1*b*) and 'Syntocinon' on 3 out of 6 chromatograms gave also an additional spot, which agrees with the statement of the makers that these preparations contain some inert material. Table 1 gives tentative R_F values for the active octapeptides.

The oxytocic activity of ox, pig and rat pituitary extracts was found in the same region of the chromatograms as that of synthetic oxytocin. The position of the pressor activity of rat gland extracts corresponded to that of arginine-vasopressin, which accords with the pharmacological evidence of van Dyke *et al.*[7], who found that the vasopressin of rat pituitaries behaves like arginine-vasopressin when injected intravenously into dogs. Ox hypothalamus yielded pressor and oxytocic activity at the R_F of arginine-vasopressin and synthetic oxytocin, but a marked discrepancy was noted between the colour intensity of the spots and the biological activity present in the eluates of the corresponding areas. For the present we cannot say whether this discrepancy is due to substances with a much lower potency than that of the glandular hormones or to the presence of inert material with very similar or identical R_F values as those of oxytocin and vasopressin.

Our results suggest that the colour intensity obtainable with the chromatographic procedure described varies considerably from one neurohypophysial peptide preparation to the other. For example, up to 1,500 mU. of synthetic oxytocin had to be applied to obtain a well-stainable spot, whereas 400 mU. of highly purified arginine-vasopressin (equivalent to about 0·7 μgm. of the pure peptide) were sufficient to give a spot of similar intensity. On the other hand, as little as 10 mU. (= approx. 0·02 μgm. peptide) of oxytocic or pressor activity could be eluted and assayed.

We are indebted to Prof. H. Konzett for providing the substituted oxytocins.

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Complex Forms of Cellulase

Recently, several articles have been published on the multiplicity of cellulase. Using paper chromatography Jermyn[1] found eight components in cellulase from *Aspergillus oryzae*, each with cellulolytic activity. Reese and Gilligan[2] found, also with chromatographic techniques, three components in *Myrothecium* cellulase, whereas Miller and Blum[3], using starch electrophoresis, found at least eight components in this product. Grimes[4] isolated from *Myrothecium* cellulase six components, of which possibly three had cellulolytic activity, and Thomas[5] found seven protein fractions by paper electrophoresis. These facts are puzzling. Whitaker[6] succeeded in isolating a homogeneous protein from crude *Myrothecium* cellulase which exhibited all the activities of the crude product, so there is a possibility that cellulase is a single enzyme. However, this has never been confirmed.

Comparing cellulase with other depolymerizing enzymes, for example, amylases and pectinases, there is a possibility that the cellulose breakdown is brought about by several discrete enzymes, a possibility which is enhanced by experiments which show that the action on cellobiose is separable from other activities[7].

That the number of discrete enzymes would in this case be as great as is indicated by the results mentioned above seems improbable.

We therefore supposed that in a crude cellulase preparation the enzyme or enzymes might be bound to a number of metabolic products, thus giving rise to a large number of complexes with cellulolytic activity.

Consequently our efforts were concentrated on the search for a method by which these bound forms of cellulase could be split up. We think we have found the first step for this purification in using a special chromatographic technique.

A crude cellulase product was obtained by growing *Myrothecium verrucaria* on a cellulose – salts medium for 14 days. Culture filtrates were concentrated in a Bartholomew evaporator, dialysed, freeze-dried, dissolved in a small amount of water, centrifuged, ultra-filtered through collodion membranes (similar to that used for dialysis) and again freeze-dried. The resulting product was a light brown powder, and normal fractionation procedures with ammonium sulphate, alcohol or acetone proved unsuccessful with respect to separation of activity and colour.

The activity of this powder was estimated, using hydrolysis of carboxy methyl cellulose as a standard.

A 50 mgm. portion was placed on top of a column of Schleicher and Schüll cellulose powder, No. 123, which had been washed previously with distilled water to which had been added sodium hydroxide to give a *p*H of 11·0 (glass electrode). The loaded column was then percolated with the same solution.

The effluent was collected in 2-ml. portions in tubes which had been filled with 2 ml. of buffer at *p*H 5·6. The enzyme activity of these was determined. Two fractions were obtained, the first containing all the coloured substances, the second a perfectly colourless solution.

The specific activity of the original material was 140 cellulase units per mgm. nitrogen. Activity of fraction 1 was 70 cellulase units, that of fraction 2 was 310 cellulase units. Preliminary dry-weight measurements on fraction 2 showed a four-fold increase in activity per mgm. dry-weight, while in

fraction 1 this was diminished by a factor of three. Overall losses were rather low, about 75 per cent of the original activity being recovered.

The method was very sensitive to small alterations in the conditions. At *p*H 5, 6, 7, 8 and 9 no separation took place. At *p*H 12 great losses occurred, presumably due to denaturation. Increasing ionic strength by addition of small amounts of sodium chloride up to 0·1μ also prevented the separation.

Cellulose as a carrier seems to be indispensable, identical experiments on starch columns being unsuccessful.

The question arises as to how far formation of complexes affects the activity of the free enzyme. When larger amounts have been separated, experiments will be carried out to elucidate these phenomena.

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A Micro Sample Introduction System for Gas Chromatography

An ideal sample introduction system for gas chromatography suitable for use with a variety of columns and detection devices should possess the following characteristics: (1) the entire sample should be delivered to the inlet of the column as a vapour over a short time-interval to keep dilution of the sample with carrier gas at a minimum; (2) the samples delivered should be of reproducible quantity or, better, of precisely known quantity; (3) the introduction of sample should not necessitate stopping or changing the flow-rate of carrier gas; (4) the quantity of air introduced with the sample should be minimal.

With the availability of a variety of high-sensitivity detectors for gas chromatography, it has become possible to perform analyses using only a few μgm. of total sample. To take advantage of this increased sensitivity of detection, without the necessity for dilution and contamination of liquid samples with relatively large quantities of carrier liquid, an apparatus permitting the reproducible injection of known volumes in the μgm.-range was designed (Fig. 1). Samples are sealed into small glass capillaries in which the volume of liquid can be accurately measured, and introduction of the sample is achieved by crushing the capillary in the gas stream at the column inlet. There is little simultaneous injection of air, and it is not necessary to interrupt or change the flow of carrier gas at the time of introducing the sample.

The internal diameters of 1-cm. lengths of finely drawn glass capillary tubing are measured with a measuring microscope. The end of the section of tubing is applied to the surface of a liquid sample and allowed to fill partially. The capillary is shaken gently until the liquid sample occupies its central section, whereupon the ends of the capillary are sealed in a flame. The length of the column of liquid

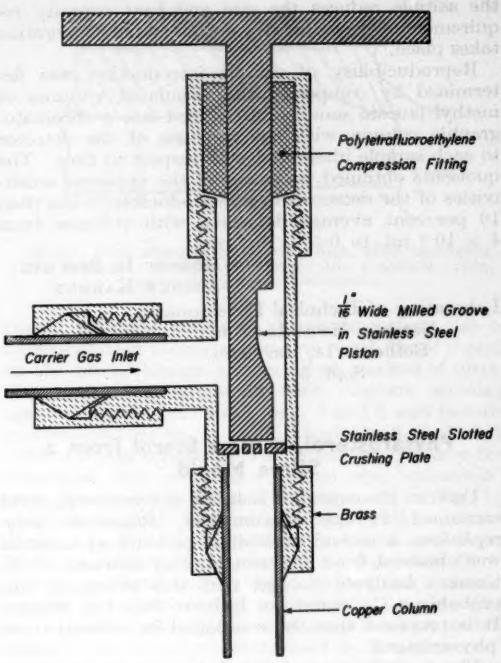

Fig. 1

is measured with a measuring microscope and the volume of liquid then calculated. A capillary of 0·1 mm. internal diameter contains $7{\cdot}8 \times 10^{-6}$ cm.³ per mm. of length. In analyses using detection devices in which insensitivity to interruption of the flow of carrier gas permits easy access to the column inlet, the capillary may be dropped and crushed at the top of the column against a slotted plate provided for the purpose. For use with detection devices in which no interruption of the gas flow-rate is desirable, several simply constructed devices for the introduction of the capillary filled with liquid sample have been designed.

The device consists of a vertical section of brass tubing with a side-arm, modified from a standard T-shaped copper tubing connector. A slotted plate is fixed in place over the column inlet at the lower end of the vertical section. Carrier gas enters the connector through the side-arm. A 1-cm. long groove is milled in the side of a stainless steel piston fitted into a sliding joint at the upper end of the vertical section. The joint is maintained leak-tight by means of a poly-tetrafluoroethylene compression fitting somewhat longer than the milled groove. In operation, the piston is moved upward to expose the groove above the fitting, the capillary filled with sample is placed within the groove, and the piston pushed down to its original position, with the groove in the gas stream. The capillary slides free of the piston coming to rest on the slotted metal plate below. The small quantity of air contained in the groove is permitted to pass through the column following which the capillary is crushed by the piston and the sample thus injected.

Because of the heat-resistant properties of poly-tetrafluoroethylene, injection of sample may be accomplished at temperatures high enough to permit rapid volatilization of the liquid. The small size of

the sample reduces the size and heat capacity requirement of the surface from which rapid evaporation takes place.

Reproducibility of sample introduction was determined by comparing the calculated volumes of methyl laurate samples introduced into a chromatographic column with the response of the detector to each sample integrated with respect to time. The quotients obtained, measures of the apparent sensitivities of the detector, were reproducible to less than 10 per cent average deviation with volumes from 4×10^{-6} ml. to $0{\cdot}5 \times 10^{-3}$ ml.

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Physarosterol, a New Sterol from a Slime Mould

During the course of isolating the yellow pigment contained in the myxomycete, *Physarum polycephalum*, a second crystalline (colourless) material was obtained from the neutral ether extracts of the tissue. Analyses showed that this substance was probably a C_{30} sterol not hitherto found in Nature. It is proposed that the compound be referred to as 'physarosterol'.

Physarosterol was isolated by separately homogenizing three different batches of tissue (15–20 gm. each) in an hydraulic homogenizer[1] with three volumes of 0·007 *M* potassium arsenate buffer[2] (*p*H 7·0). The homogenate was acidified with sulphuric acid and a one-third volume of alcohol was added. After addition of filter aid, the slurry was filtered by vacuum. The yellow filtrate was saturated with sodium chloride, filtered, and continuously extracted with ether until no more pigment was carried over into the solvent. Then the ether extract was concentrated to 100 ml. at 60° C., made alkaline with 4 *N* sodium hydroxide solution and again extracted five times with ether (separatory funnel). The aqueous phase which contained the yellow pigment was stored for later processing. The nearly colourless ether solutions were combined and allowed to evaporate slowly at room temperature. Gradually a layer of lipid formed on the top of the solution and crystals began to be deposited on the bottom of the vessel.

When crystal formation had ceased, the lipid and underlying solution were decanted and the crystals removed by filtration. Approximately 25 mgm. of sterol were obtained from 100 gm. of tissue. Recrystallization was effected twice from a minimum volume of warm alcohol–ether (1 : 2). The crystals were dendritic leaves. The following analysis was obtained: calculated for $C_{30}H_{52}O_3$: C, 78·21; H, 11·39. (Calculated for $C_{30}H_{50}O_3$: C, 78·54; H, 10·98); found: C, 78·17; H, 11·31 $[\alpha]_D^{26}$ −55·3° (*c.* 0·5 in chloroform), melting point 137–139° C. (Fischer Johns apparatus).

The compound gave an immediate digitonide precipitate and an immediate lavender colour in the Lieberman–Burchard test (eventually a green precipitate was formed). Concentrated sulphuric acid produced an intense orange colour. Physarosterol gave no colour either with tetranitromethane or with ferric chloride. A bromine solution was immediately decolorized by a solution of the sterol in glacial acetic acid. A test for alkoxy groups was negative. No carbonyl band was observed in the 260–290 mμ region of the ultra-violet (*c.* 23 mgm./10 ml. in chloroform).

The closest fitting empirical formula for physarosterol ($C_{30}H_{52}O_3$) would allow for the presence of five-ring structures. The *ABCD* rings of the cyclopentenophenanthrene nucleus would account for four of these rings. In the absence of a carbonyl group, a fifth carbocyclic or methylene dioxy ring could be excluded because the evidence for a double bond accounted for the fifth ring structure. Furthermore, since the tetranitromethane test was negative, the double bond may have been sterically hindered and endocyclic.

Two attempts were made to acetylate physarosterol by exposing it to acetic anhydride and pyridine at room temperature for 24 hr. In both experiments, the parent compound was recovered unchanged. This difficulty suggested that the hydroxy groups were in juxtaposition to tertiary carbon atoms.

In summary, these results indicated that physarosterol was a C_{30}, unsaturated, trihydroxy sterol with one of its hydroxyl groups in the 3β position.

Because the isolation of this substance is incidental to other aspects of slime mould chemistry at present being pursued, it is not anticipated that any further work will be carried out in this laboratory in regard to its structure.

I am indebted to Mr. Ralph Karler of this department for providing generous samples of *Physarum polycephalum*.

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Inhibition of Pancreatic Deoxyribonuclease by Acidic Polymers

In a recent publication the inhibition of pancreatic ribonuclease by a series of polymers possessing phenolic and carboxylic or sulphonic acid groups was compared with their action against influenza and vaccinia viruses in embryonated eggs. This communication describes the action of certain of these polymers on pancreatic deoxyribonuclease (Worthington Biochemical Corporation).

Enzyme activity was determined by the increase in optical density at 260 mμ of enzymatic digests at 29° ± 0·2° C. (Kunitz's method) in the presence or absence of graded inhibitor dilutions. The rates were computed from the linear portions of density – time graphs by the method of least mean squares, and by interpolation the inhibitor concentration reducing the rate to one-half of the control value (IC_{50}) was determined. The method permitted a reproducibility of rate measurements of ± 5 per cent in duplicate samples. While the absolute rate of the deoxyribonuclease reaction varied from day to day, this had no bearing on the IC_{50} values, which are relative figures, and were reproducible to ± 15 per cent or better. Table 1 shows the experimental conditions.

Table 1. Deoxyribonucleic acid solution (Worthington Biochemical Corporation): 400 μgm./ml. in 0·1 *M* acetate *p*H 5, 0·005 *M* magnesium sulphate, centrifuged and filtered. Deoxyribonuclease solution: 200 μgm./ml. in 0·1 *M* acetate *p*H 5

	Non-inhibited reaction (control)		Inhibited reaction	
	Blank	Digest	Blank	Digest
DNA solution	1 ml.	1 ml.	1 ml.	1 ml.
Water	4 ml.	3·5 ml.	3·5 ml.	3·0 ml.
DNase solution	—	0·5 ml.	—	0·5 ml.
Inhibitor ,,	—	—	0·5 ml.	0·5 ml.

Eighteen selected polymers were tested, representative of the following general types of polymerization: (1) phenolic carboxylic or sulphonic acids condensed with aldehydes; (2) polymers arising from the products of (1) on further condensation with phenolic acids under oxidizing conditions giving 'triphenylmethane' types; (3) oxidative polymerization of phenolic or hydroquinone-like compounds. In general, the half-inhibitory concentration for deoxyribonuclease was about three times that found for ribonuclease, under the conditions employed. The most notable exception was sodium humate from lignite, for which the ratio of IC_{50}-deoxyribonuclease to IC_{50}-ribonuclease was about 13. For the sake of comparison, heparin, sodium chitin sulphate and the polyurea from 4,4′-diaminodibenzyl-2,2′-disulphonic acid were also examined and found to agree with the above generalization, while suramin appeared to be weakly inhibitory at low, and seemingly accelerating at higher concentrations. The results for a few representative polymers are presented in Table 2. The results indicate that no noteworthy qualitative differences exist between the responses of the two nucleases to the acidic polymers investigated.

Table 2. Inhibition of Ribonuclease and Deoxyribonuclease by Acidic Polymers
(IC_{50}, half-inhibitory concentration)

Polymer	IC_{50} ribonuclease μgm./ml.	IC_{50} deoxyribonuclease μgm./ml.
Gentisic acid + CH_2O	2	4
Gentisic acid + $K_2S_2O_8$ + NH_3	3	10
(Gentisic acid + CH_2O) + HNO_2 + gentisic acid	1	3
Hydroquinone sulphonic acid + CH_2O	13	32
Anthranilic acid + $FeSO_4$ + H_2O_2	5	17
Hydroquinone + $K_2S_2O_8$	2	6

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Electron Microscope Studies of the Chemical Reactivity in Keratin Cuticle

Detailed information about the structure of a cuticular layer of flat overlapping cells, covering animal hairs and wools, is of great technological importance. Its ratchet-like character is responsible for the greater frictional resistance to motion in the tip to root than the root to tip direction; and is one of the causes of felting.

The multilayer structure of an individual cuticle cell has been clearly established: on one hand, indirectly, from experiments involving mechanical and/or chemical fragmentation[1–5], and closely supported by some results of the work making use of high-resolution atomic replicas of wool fibres[6]; and, on the other, through a more direct method of ultra-thin sectioning, combined with electron staining agents (osmium tetroxide) (refs. 7 and 8 and private communications by A. O. T. Charles and H. Mahl).

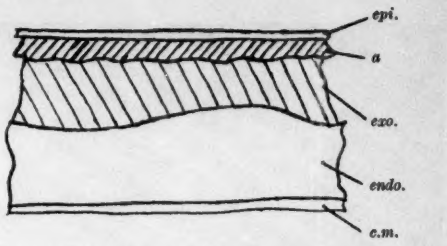

Fig. 1. Layer structure of keratin cuticle, after Lagermalm. *Epi.*, epicuticle; *a*, highly inert layer; *exo.*, exocuticle; *endo.*, endocuticle; *c.m.*, cell membrane

Thus it was concluded that immediately below the outermost thin membrane (called the 'epicuticle') there lies an easily stained thin layer (*a* in Fig. 1). resistant to both keratinolytic agents and enzyme digestion. This is followed by a keratinized layer (the 'exocuticle') which, in a fully hardened fibre, appears to show only a slight differential staining, relative to the lower cytoplasmic layer (the 'endocuticle'—insoluble in keratinolytic solvents, but attacked by enzymes), attributed to a higher concentration of sulphur in the former[8]. The character of the layer *a* is not clear; however, there can be little doubt that such a layer, if indeed present, will play an important part in determining the stability of fibres to the action of chemical agents, or such mechanical treatments as may be used to modify the frictional properties of wool in order to reduce the rate of felting.

In all these considerations an assumption was made that osmium tetroxide reacts strongly with cysteine or cystine[9]; and it occurred to us that, in order to obtain an independent confirmation of this view, use could be made of some metal salts known to form (coloured) metal sulphides in keratin[10–15].

The present investigations involved merino and Lincoln wools, mohair fibres, human hair, pig bristle and porcupine quill, which were treated either in mercury or lead salts[14,15]. In the latter case, keratin specimens were treated, for a period of up to 18 hr., in aqueous sodium plumbite solutions, at 35° C. and *p*H 11·8, at a concentration equivalent to 0·1 per cent lead nitrate[14]. Following this treatment, the specimens were washed for 12 hr., first in water at 35° C. and then in running cold water. The ultra-thin sections (about 200 A. in thickness) were obtained on a commercial Philips ultra-microtome (fitted with a glass knife, 45°), using 'Araldite' as an embedding medium[16].

Fig. 2 shows the differentiation of layers in the cuticle of pig bristle, in agreement with the scheme of Fig. 1, but more clearly than in the experiments involving osmic acid (refs. 7 and 8 and private communications by A. O. T. Charles and H. Mahl). X-ray diffraction photographs of the bulk of fibres confirm the formation of lead sulphide, and there can be little doubt that the dark spots visible throughout the cross-section are related to this compound.

We are aware that some aspects of the detailed interpretation of results obtained by this method are still uncertain (such as a possibility of migration

Fig. 2. An electron micrograph of a cross-section of pig bristle, after treatment in sodium plumbite. Note: the outermost cuticle cell, in top left-hand corner; and the cortex, in bottom right-hand corner. Direct print from the original (× c. 20,700)

during the formation of metal sulphides), and further experiments are in progress to clarify this and other points. Nevertheless, we believe that, even at this early stage, the workers in this and some allied fields may be interested to have their attention directed to the efficacy of our method of detecting the distribution of active groups in keratin with heavy metals, providing, thereby, a greater degree of electron contrast (though qualitatively estimated at present) than that obtained with metal atoms normally used in the practice of electron staining[17].

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A Galactan from *Mycoplasma mycoides*

Little is known of the carbohydrates of *Mycoplasma mycoides* var. *mycoides*, the organism responsible for bovine contagious pleuropneumonia. (This is the nomenclature used by R. S. Breed, E. G. D. Murray and N. R. Smith in the seventh edition of "Bergey's Manual of Determinative Bacteriology", p. 1. (Williams and Wilkins Co., Baltimore, 1957). We use the abbreviation *M. mycoides* in what follows. The organism has been widely known in the past as *Asterococcus mycoides*.) Kurotchkin[1], however, was able to isolate a serologically active fraction which gave positive Molisch and negative biuret reactions. We have examined the strain (*V5*) of *M. mycoides* used in this laboratory for the preparation of bovine contagious pleuropneumonia vaccine, with the object of obtaining material which might be of value in immunological studies.

The organisms were grown in *BVF-OS* medium[2] inoculated with 5–8 ml. of a 4-day culture per litre. After incubation of 24–28 hr. in rotated flasks at 37° C. they were harvested in a steam-driven Sharples supercentrifuge and washed in cold 0·2 *M* sodium chloride, and in cold distilled water. When the washed organisms were hydrolysed in 0·25 *N* sulphuric acid and the hydrolysates chromatographed in four solvent systems, galactose and ribose were the only sugars detected. Glucose, if present, amounted to less than 3 per cent of the total hexose. The identity of galactose was confirmed by preparation of the α-methylphenylhydrazone. No evidence for the presence of amino-sugars, uronic acids or heptoses could be obtained by the application of colorimetric tests[3]. The washed suspensions were found to contain approximately 10 per cent of their dry weight as carbohydrate when analysed by the anthrone procedure of Trevelyan and Harrison[4] using galactose as the standard.

Washed suspensions were frozen and thawed three times, buffered to *p*H 8 by the addition of *tris*-hydroxymethylaminomethane to a concentration of 0·04 *M*, and centrifuged at 26,000*g* for 20 min. in rotor 40 of the 'Spinco' preparative ultra-centrifuge model *L*. The galactose was almost quantitatively sedimented while most of the ribonucleic acid remained in the supernatant. The pellet was extracted with warm aqueous phenol according to the method of Westphal *et al.*[5]. The aqueous phase contained a polysaccharide together with some ribonucleic acid. This solution was dialysed, made 0·1 *M* with respect to acetate (*p*H 8) and centrifuged at 105,000*g* for 4 hr. The polysaccharide sedimented more rapidly than the ribonucleic acid, and was almost completely freed of the latter after two or three centrifugations. Ribonucleic acid could also be removed by passing the dialysed aqueous solution through columns of 'Dowex-1' chloride (2 per cent cross-linked). This method was more convenient than ultracentrifugation and gave higher yields.

The polysaccharide contained small and variable amounts of nitrogen (0·2–0·8 per cent) and traces of phosphorus (< 0·1 per cent). After hydrolysis in 0·5 *N* sulphuric acid for 2 hr. at 100° C., 85–89 per cent of the material was accounted for as reducing sugar (calculated as anhydrohexose). Galactose was the only sugar detected on paper chromatograms. The polysaccharide was not pyrogenic when injected into rabbits. It gave positive precipitin reactions with *M. mycoides* antisera, sensitized erythrocytes to

agglutination by the antisera and itself inhibited the agglutination of sensitized erythrocytes by the antisera (Cottew, G. S., unpublished observations). It thus has the properties of a complex hapten. No antibody formation could be detected following injections of the galactan into rabbits. Its relationship to the soluble antigens recently reported by White[6] is not known.

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Formation of Ketones from Fatty Acids by Spores of *Penicillium roqueforti*

From time to time during the past decade attempts have been made in this laboratory to determine the mechanism by which the mould *Penicillium roqueforti* produces the flavouring and aromatic compounds characteristic of the blue-veined cheeses, Roquefort, Stilton, Gorgonzola and 'Bleu'. These aromatic compounds are a family of ketones, each one produced from a specific fatty acid which is one carbon longer, the carboxyl carbon, than the ketone. Thus the mould converts caprylic (octanoic) acid to 2-heptanone, caproic (hexanoic) acid to 2-pentanone, and so on. The overall kinetics for the conversion of fatty acids to ketones by *P. roqueforti* was published in 1955[1]; however, the individual steps in the conversion were not known. Until recently, the occurrence of the reaction under controlled conditions in the laboratory has been most erratic and unpredictable, not at all commensurate with the apparent reliability of the reaction in the manufacture of blue-veined cheese.

In attempts to study the reaction, young actively growing mould cells have always been used since, in all our work on the metabolism of filamentous fungi, such cells have much higher levels of metabolic activity. However, after a time it became apparent that the older cells were somewhat more reliable in ketone production and that the medium in which the cells were grown considerably influenced the reaction. A completely synthetic medium that provided excellent cell growth with no sporulation in submerged culture was developed[2], but mould cells grown on this medium completely lacked the ability to form 2-heptanone from octanoic acid. The formation of 2-heptanone has been used as a measure of the overall ability of the cells to form ketones from fatty acids and a chemical assay for 2-heptanone has been devised. Small amounts of corn steep liquor, protein hydrolysates and certain amino-acids when added to the synthetic medium caused the cells to sporulate, but these cells rapidly formed 2-heptanone when octanoic acid was added; it soon became evident that sporulation was a requisite for ketone formation and that the rate of ketone formation was directly proportional to the degree of sporulation. Even more surprising was the finding that it was indeed the spores that were forming the ketone and not the mycelium. Studies on the kinetics of the reaction using spores separated from the mycelium by filtration through pads of glass-wool show a direct relationship between oxygen uptake and ketone formation; all the oxygen taken up is used in ketone formation and the spores do not germinate. Spores that have been allowed to germinate or form even the smallest germ tubes cannot form ketones. Details on the kinetics and stoichiometry of the conversion of fatty acids to 2-ketones will be published elsewhere.

These findings implicate the spores of *P. roqueforti* in a series of reactions seemingly quite removed from dormancy and germination. Immediately one wonders if other mould spores, like these, might not have physiological possibilities that are now unknown or are ascribed to the vegetative cells.

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Biological Potency of Eta-Tocopherol

η-Tocopherol was first isolated from rice by Green and Marcinkiewicz[1]. DL-η-Tocopherol has been synthesized by McHale *et al.*[2]. In a preliminary report to the fourth International Congress of Nutrition, Bunyan *et al.*[3] gave the potency of η-tocopherol as less than 5 per cent of α-tocopherol.

A full-scale gestation resorption assay of DL-η-tocopherol has now been completed. Virgin Norwegian hooded rats, depleted of vitamin E for five to seven months, were mated and received oral doses in equal portions on the seventh and eighth days of pregnancy, being dissected on the eighteenth day. Results from rats with less than five implantation sites were ignored and the presence of two live fœtuses was taken as the minimum positive response. Forty-five dosed rats provided the assay results, and eight undosed controls all gave negative responses. DL-η-Tocopherol was found to have 3 per cent of the potency of DL-α-tocopheryl acetate (limits of error 0·5–5·4 per cent, $P = 0·05$).

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Differences between Duplicate Assays of Human Urinary Gonadotrophins

Sheps and Munson[1] have shown how the internal statistics of a single hormone assay may underestimate the variation actually observed on replication of that assay. Inter-assay errors may therefore be important when demonstrating qualitative differences between two hormone preparations by the simultaneous use of two different methods of assay. Such differences have been shown in human urinary gonadotrophins[2], and the errors between duplicates of the assays used have therefore been investigated.

The assays were for (*a*) total gonadotrophin, using the uterine response in immature mice, and (*b*) follicle-stimulating hormone, using the ovarian response in similar mice treated with excess human chorionic gonadotrophin[3]. Pairs of extracts obtained from human urine by the kaolin–acetone method were selected only on the grounds of availability and the fact that they had previously been assayed against one another. They were then re-assayed under the conditions described below, which were arranged so that inter-assay errors were likely to be maximal.

The coded solutions were made up by the same person but were assayed by different persons on the two occasions. In the second assay the dose-levels were designed to be as different from the original ones as was practicable. In five pairs of assays for total gonadotrophin the initial assay was calculated from quantal data and the second from the graded response. From one to seventeen months elapsed between the initial and second assays to allow for effects of storing the extracts. In assaying follicle-stimulating hormone a different batch of chorionic gonadotrophin was used in each assay of a pair.

The results of the assays using graded responses were calculated using log organ weight as the response metameter. Examination of the untransformed results pooled for each method of assay showed an obvious correlation of variance with response in both. The use of log-response stabilized variance satisfactorily in both assays, and such a transformation seems desirable in calculating the results of these assays.

Tables 1 and 2 show respectively the results of ten pairs of assays for total gonadotrophin and ten pairs for follicle-stimulating hormone. In the majority of the duplicate assays the results agree well. The fiducial limits ($P = 0{\cdot}95$) are similar in range to those which have to be used in practice[2], and in two of the twenty cases the limits of the two estimates do not overlap. These cases show significant inter-assay errors and suggest that the failure of the limits to overlap should not be used alone as evidence of significant differences between the results of two assays of a gonadotrophin. The degree of difference of the best estimates must also be taken into account

Table 1. Duplicate Assays of Total Gonadotrophin

Pair	Months between assays	Number of dose-levels 1st	2nd	Percentage potency (95 per cent fiducial limits) 1st	2nd	Ratio of potency estimates
1	1	2+2	2+3	72 (48–96)	92 (65–125)	1·28
2	1	2+2	2+2	348 (296–408)	238 (208–274)	1·46
3	4	2+2*	3+3	80 (49–115)	72 (53–119)	1·11
4	2	2+2	3+3	84 (56–117)	79 (43–116)	1·06
5	2	2+2	2+3	119 (70–232)	96 (74–124)	1·24
6	15	3+3*	3+3	82 (63–112)	71 (54–90)	1·15
7	15	3+3*	2+3	23 (17–31)	11 (7–15)	2·09
8	1	2+2	3+3	51 (34–72)	57 (51–69)	1·12
9	13	3+3*	2+2	114 (86–167)	100 (85–133)	1·14
10	13	3+3*	2+3	75 (52–114)	59 (35–76)	1·27

* Calculation from quantal data.

Table 2. Duplicate Assays of Follicle-Stimulating Hormone

Pair	Months between assays	Number of dose-levels 1st	2nd	Percentage potency (95 per cent fiducial limits) 1st	2nd	Ratio of potency estimates
11	2	2+2	2+2	292 (176–440)	154 (32–270)	1·90
12	11	2+3	3+3	27 (20–38)	29 (17–49)	1·07
13	11	2+3	2+3	17 (12–23)	9 (4–16)	1·89
14	10	2+3	2+3	986 (694–1,370)	735 (508–971)	1·34
15	12	2+3	2+2	848 (633–1,140)	877 (763–1,040)	1·03
16	1	2+3	2+3	264 (168–413)	266 (140–440)	1·01
17	4	2+2	3+3	1,620 (952–3,920)	1,810 (1,260–2,740)	1·12
18	17	2+2	2+3	67 (34–114)	95 (68–113)	1·42
19	2	3+3	3+3	15 (10–22)	15 (6–27)	1·00
20	8	2+2	3+3	2,600 (1,780–4,880)	4,800 (2,790–11,700)	1·85

and in only one case in these twenty duplicates does the ratio of the two estimates reach a value of two.

The magnitude of the inter-assay error is roughly similar for the two methods. It is therefore likely that when a gonadotrophin is assayed by each method a similar error will occur between the two estimates of potency. The conditions of the present experiment were designed to accentuate inter-assay errors and a two-fold difference in estimates occurred only once in twenty pairs. If, therefore, the two methods of assay under normal conditions give estimates which show a more than two-fold difference, and the fiducial limits of which do not overlap, it is very likely that these results demonstrate a genuine qualitative difference. This is being used as a rough working principle until more precise analyses of these and similar data are available.

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Concentration in the Human Ovarian Follicular Fluid of Radioactive Tracers and Drugs circulating in the Blood

The potential hazard of genetic changes induced by radioactive substances and of mutagenic and radiomimetic action of non-radioactive compounds is of current interest. Information, however, as to the intimacy of contact of these substances, when introduced into the human body, with the reproductive cells, particularly the ovum, is scant.

It has been well established that many radioactive tracers, compounds and drugs are transferred from the vascular into other compartments of the human body. However, it is not known whether in the human these substances are transferred into the ovarian follicular fluid. Since the follicular fluid surrounds the ovum and corona radiata and the egg might depend for its nutrition on this material, a transfer of exogenous substances into the ovarian

follicular fluid might bring potentially noxious material into close proximity with the ovum or, by means of the cells of the corona radiata, into actual contact with the egg.

Previous animal studies[1–5] with sulphur-35 and to a lesser extent[6] with phosphorus-32, as well as with the dyes, trypan blue[7] and Evans blue[8], have shown the appearance of all of these compounds in the ovarian follicular fluid. Thus, beta-emitting radioisotopes have already been shown in animals to accumulate in an area where they may potentially do harm. Damage to the offspring has been described when the ovaries of intact animals were briefly exposed to a carcinogenic substance[9].

Our particular interest in this problem stemmed from our observations in a related field—intravenously administered iodine-131 and sodium thiocyanate-^{35}S, were rapidly excreted with and concentrated in the human cervical mucus. Their concentrations in mucus were many-fold higher than those obtained in blood[10]. As the next step in this investigation of the accumulation of exogenous material within the human female genital tract, the possible appearance of such material in the follicular fluid was investigated.

These studies were performed in twenty-nine premenopausal women undergoing hysterectomy, the principal indications for this procedure being cystocœle, menometrorrhagia or myomata. Eight patients received two different drugs simultaneously. None showed any gross abnormality of ovarian structure or function. The ovaries were exposed during surgery and the follicular fluid aspirated by puncturing a prominent follicle with a 25-gauge hypodermic needle attached to a tuberculin syringe. A venous blood specimen was drawn simultaneously. Whenever possible, a segment of the punctured follicle was removed for histological examination. Follicular fluid and blood were examined immediately. The following materials—iodine-131, iodine-131-tagged human gamma-globulin, mercury-203-tagged mercaptomerin, carbon-14-tagged meprobamate, penicillin and 'Pyridium' were given before hysterectomy by the route and at the time-intervals indicated in Table 1.

Table 1. The Relative Concentration in Human Ovarian Follicular Fluid and Serum of Radioactive Tracers and Drugs

Drug and route of administration	No. of observations	Interval at which specimen obtained (hr.)	Ratio: Follicular fluid / Serum	
			Mean	Range
^{131}I (intravenous)	7	0·25–7	0·44	0·14–0·97
^{131}I (intravenous)	7	12–60	1·13	0·60–1·62
^{131}I-human gamma-globulin (intravenous)	6	13–21	0·45	0·09–0·81
^{203}Hg-mercaptomerin (intravenous)	3	11–12	14·18	4·20–19·70
^{14}C-meprobamate (per os)	4	2·5–11	1·03	0·59–1·40
Penicillin (intramuscular)	4	2–4·5	0·33	0–0·50

Iodine-131 and mercury-203 activity were determined by gamma counting with a well-type scintillation counter, and carbon-14 was measured by beta-assay with a windowless flow counter. Penicillin concentration was determined from the bacteriostatic activity of the samples. Qualitative tests were employed to detect the presence or absence of 'Pyridium' in the follicular fluid. The results are expressed as the ratio of the relative concentrations of the test materials in the follicular fluid and serum.

The results show that all the compounds tested, iodine-131, iodine-131–gamma-globulin, mercury-203–mercaptomerin and carbon-14–meprobamate, penetrate from the blood stream into the human ovarian follicular fluid (Table 1). The results from the studies with iodine-131 show that penetration commences within 15 min., and the results with iodine-131–gamma-globulin suggest that a large molecule can pass into the follicular fluid at a slower rate. Of particular interest is the appearance in the follicular fluid of a mercurial diuretic agent and of a tranquillizer. 200 mgm. of 'Pyridium' was given orally 3–19 hr. before puncture of the follicle in six patients; the drug was detected in the follicular fluid in every instance.

Sixteen histological preparations were obtained; six of them revealed fresh corpora lutea from which tertiary follicular fluid had been obtained. The results are not sufficiently extensive to show any difference in the concentration of substances in the fluid from normal follicles as compared with that from the young corpora lutea. Our previous observations[10] have shown, however, that the ratio for iodine-131 is very low for large pathological ovarian cysts.

The results indicate not only that administered compounds penetrate into the human ovarian follicular fluid but that they may be concentrated therein. This property might be particularly significant when exhibited by drugs with mutagenic and radiomimetic effects or with the capacity to interfere with enzyme systems of the ovum, particularly during division. It is not known whether drugs present within the follicular fluid can do harm to the ovum, especially when administered over a long period of time, or at a particular stage in its development, such as when the ovum is dividing.

These studies suggest that the human egg *in situ* within its own follicle is exposed to therapeutic agents introduced into the general circulation. We are at present attempting to determine whether such materials appearing in the ovarian follicular fluid actually penetrate into the egg proper.

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Effect of Nitrous Oxide on the Radiosensitivity of Mouse Ehrlich Ascites Tumour

It has been shown that inert gases at pressures higher than 1 atm. reduce the oxygen-dependent radiosensitivity of the broad bean (*Vicia faba*) root and of mouse Ehrlich ascites tumour cells in the presence of oxygen[1]. The effectiveness of the inert gases follows their partition between water and lipids and is paralleled by their anæsthetic potency. It therefore seemed of interest to test nitrous oxide. Results obtained with nitrous oxide on mouse Ehrlich ascites tumour cells show a marked effect, and, since this gas is effective in the range physiologically tolerated by mammals, it seems worth while reporting the results (Table 1).

Table 1

Experiment	Gas mixture during exposure to X-radiation	Dose in r. to reduce normal anaphases to 31·6 (per cent)	Relative radiosensitivity
1	1 atm. of air	130 ± 10	3·5 ± 0·09
2	0·2 atm. of oxygen + 0·8 atm. of nitrous oxide	500 ± 20	0·91 ± 0·02
3	1 atm. of air + 95 atm. of argon	455 ± 20	1·0 ± 0·04
4	1 atm. of air + 11 atm. of nitrogen	330 ± 15	1·4 ± 0·02
5	1 atm. of air + 2 atm. of xenon	380 ± 15	1·2 ± 0·02

The experimental techniques and the method of evaluation of the results are those described previously[1], except that in Experiment 2 the tumour was fixed 12 hr. after irradiation because the maximum damage occurred then and not after 18 hr., as was the case in the other experiments.

It can be seen from Table 1 (Experiment 1) that the relative radiosensitivity of mouse ascites tumour cells, when exposed to 190-kV. X-radiation in 1 atm. of air, that is, 0·2 atm. of oxygen and 0·8 atm. of nitrogen, was 3·5 ± 0·09. The addition of 0·8 atm. of nitrous oxide to 0·2 atm. of oxygen (Experiment 2) reduced the radiosensitivity to the same extent as 95 atm. of argon (Experiment 3), that is, to the same low level as observed in irradiation under nitrogen alone[1]. Table 1 also shows the results of experiments carried out with the mixtures 1 atm. of air plus 11 atm. of nitrogen (Experiment 4), and 1 atm. of air plus 2 atm. of xenon (Experiment 5). These indicate that nitrous oxide is the most effective of the gases tested in blocking the effect of oxygen on the radiosensitivity of Ehrlich ascites tumour cells.

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Acclimatization Status of Temperate-zone Man

When men accustomed to living in a temperate climate are repeatedly or continuously exposed to a natural or artificial hot environment they rapidly develop a greatly increased capacity to withstand heat, and this adaptation is accompanied by profound and readily demonstrable physiological changes. On the other hand, when men are similarly transferred from a temperate to a cold, even arctic, environment neither do they display the same rapidly increased tolerance of their new environment nor has it yet been found possible to demonstrate comparable profound physiological changes. Such marked changes as have been demonstrated are, in general, local adaptations, such as the diminution in finger numbness described by Mackworth[1].

This is a puzzling state of affairs, because it might seem logical to expect that men would possess an ability to adapt to cold comparable to their ability to adapt to heat. The difficulty appears to arise from the assumption that a temperate climate represents the neutral state in which environmental stress is at a minimum and that divergence from it, either to cooler or to warmer conditions, is accompanied by an increasing physiological strain.

In order to determine the normal or neutral condition for man he must be considered as an animal; that is, in a situation in which, devoid of clothes, fire and shelter, he is able to exist without undue physiological strain both during the day, when the very moderate energy expenditure of food-gathering is punctuated by occasional high rates of expenditure in such crises as flight or combat, and during the inactive hours of darkness and sleep. In such circumstances it would appear that man is best suited to the warm, equable environment of a tropical forest, with its small diurnal variation in temperature and the protection it affords from heat gain from solar radiation during the day and heat loss by radiation to a clear sky at night.

As Martin[2] and others have shown, considerable physiological evidence can be marshalled in support of this view. Man's scanty hair provides little insulation. His capacity for prodigious cutaneous vasodilatation and his unequalled ability to sweat (which are not matched by comparable mechanisms for heat conservation) together provide a means for heat loss adequate, even in a warm climate, not only for his usual moderate rate of energy expenditure but also for the high rates of relatively brief emergencies. The critical temperature for naked man (25–27° C.) is at the upper end of the range for tropical animals[3], which means that he requires a high nocturnal environmental temperature for undisturbed sleep. Macintyre[4] has stated that the temperature between the sheets of an occupied bed in Britain is usually between 27 and 29° C. within a few inches of the body.

The most convincing evidence, however, concerning the position of the neutral state of thermal adjustment is provided by direct experimental investigation. It has been shown that European men previously adapted to life in a temperate climate display, after a period of some eighteen months residence in Singapore, an ability to work in the heat that is markedly superior to that of similar men living in England[5], and equal to that possessed by the indigenous Malay, Chinese and Indian inhabitants[6]. Such Europeans naturally acclimatized to a tropical environment do not, however, possess their maximum adaptation to heat, for exposure to higher temperatures in a climatic chamber rapidly induces a much-enhanced heat tolerance[7]. It is clear that those living in the tropics are capable of a considerable range of adaptation both to hotter and to colder conditions.

It is to be concluded, therefore, that a temperate climate does not represent the neutral condition in which environmental stress is minimum and from which adaptation is possible both to hotter and to colder conditions to an equal degree. On the con-

trary, man in a temperate climate is approaching the extreme range of adaptation to cooler conditions —that is, maximum adaptation to cold. It follows that it is fruitless to attempt to demonstrate profound physiological changes in temperate man on exposure to severe cold because, the greater part of the possible adaptation in this direction having already been made in a temperate climate, any further changes must necessarily be small.

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Extra-Regional Oocytes in Teleosts

The occurrence of extra-regional oocytes has been described by several authors in many species of vertebrates[1,2]. Most of these cases related to the genital and the caudal region.

We have observed extra-regional oocytes in the following species: *Phenacogrammus interruptus*, *Chaetodon capistratus*, *Abramites microcephalus*, *Ballistes undulatus*, *Botia modesta*, *Cottus gobio* and *Barbus gelius*. These cases are summarized in Table 1. In general, the cells had the characteristic oocyte structure, namely, granular cytoplasm, oolemma, nucleus with several nucleoli.

The presence of extra-regional oocytes is closely related to the migration of the primordial germ cells. Obviously certain cases may provide important data on the mechanism of the migration.

For the three major theories relating to the migration of the primordial germ cells we refer to the publication of Johnson[2], who observed an amœboid movement in the germ cells of the black bass. Newth[3] stated that in Lacertilians the migration of the primordial germ cells to the gonadal ridges occurs by a definite route. This type of migration has already been known for a long time in lizards and in *Sphenodon*.

Concerning our case of extra-regional oocytes in the brain of *Abramites* it may be difficult for the primordial germ cells to reach the brain (mesencephalon) by Johnson's[2] first two types of migration, for this would involve crossing a great number of obstacles and carrying the cells a long distance. Like the extra-regional oocytes in the brain of *Cirrhina reba*[4], it is therefore very likely that in this case the migration of the germ cells takes place via the vascular route as in *Sphenodon*, the Lacertilians and the birds. These oocytes might have been lost while they traversed the brain region during their migration, after which they could settle in the brain tissue.

The case of the lordotic *Botia* suggests that the presence of the extra-regional oocytes and the degeneration of these cells are a consequence of the embryological disturbances in the caudal region. It is possible that these processes have passed to abnormal places.

It is noteworthy that in the case of *Ballistes* nucleolar extrusion was observed. The tumour in *Barbus* shows that migrated extra-regional oocytes which have migrated can proliferate under certain circumstances.

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Table 1. Cases of Extra-regional Oocytes in Teleosts

Species	Localization of the oocytes	Extra-regional oocytes	Nuclei of the extra-regional oocytes	Remarks
Phenacogrammus interruptus (length 69 mm.)	Intermuscular connective tissue of the caudal region	Small, medium-sized and large types; infiltration of some large oocytes by strands of connective tissue	Several nucleoli	Externally no peculiarities
Chaetodon capistratus (length 78 mm.)	Intermuscular connective tissue of the caudal region	Medium-sized and large types; some oocytes degenerated	Several nucleoli	Externally no peculiarities
Abramites microcephalus (length 53 mm.)	Brain (mesencephalon)	Small and medium-sized types, sometimes attached to the brain tissue; infiltration of some medium-sized oocytes by strands of nervous tissue	Several nucleoli	Externally no peculiarities
Ballistes undulatus (length 83 mm.)	Intermuscular connective tissue of the caudal region	Small, medium-sized and large types	Several nucleoli, sometimes nucleolar extrusion	Externally no peculiarities
Botia modesta (length 89 mm.)	Intermuscular connective tissue of the caudal region	Small and medium-sized types; some oocytes degenerated but not infiltrated by strands of connective tissue	Several nucleoli	Lordosis
Cottus gobio (length 241 mm.)	Intermuscular connective tissue of the caudal region	Small, medium-sized and large types	Several nucleoli	Externally no peculiarities
Barbus gelius (length 70 mm.)	Caudal region	No true extra-regional oocytes, probably developed to granulosa cell tumour	—	Irregular, nodular tumour

Conversion of Carbon-14 Dioxide to Starch Glucose during Photosynthesis by Spinach Chloroplasts

The distribution of carbon-14 in the glucose moiety of sucrose, glucose phosphate and starch formed from carbon-14 dioxide during short-time photosynthesis in *Chlorella* and the leaves of higher plants has been shown to be asymmetric[1]. It was proposed that either glucose was not formed by the condensation of two

triose phosphates of similar tracer distribution or that a pool of unlabelled dihydroxyacetone phosphate was present in the cell, causing a dilution of the upper three carbon atoms of the hexose. To test the validity of the latter suggestion, the intact spinach chloroplast free of much cytoplasic material was employed.

In agreement with Allen *et al.*[2], a glucose polymer was the only insoluble product formed under the conditions used. In the 4-min. experiment, the insoluble fraction accounted for 3 per cent of the tracer assimilated while in the 30-min. photosynthesis this fraction increased to approximately 20 per cent.

All the glucoses formed by the spinach chloroplasts during these time periods possess an asymmetric distribution of carbon-14 (Table 1).

Table 1. The Distribution of Carbon-14 in Starch Glucose formed by Spinach Chloroplasts during Photosynthesis in Carbon-14 Dioxide

Exp. No.	Time of exposure	Tracer content of carbon atom number (mμc./mgm. carbon)					
		1	2	3	4	5	6
1	3 min.	0·04	0·01	0·09	0·17	0·05	0·04
2	4 min.	1·02	0·69	1·54	2·42	0·46	0·55
3	30 min.	1·57	1·40	2·14	2·33	1·14	1·10

Chloroplasts were isolated in 0·35 *M* sodium chloride[3]. Unwashed chloroplasts were used in Exps. 1 and 2. Chloroplasts once washed with 0·35 *M* sodium chloride were used in Exp. 3. Warburg vessels (150 ml. capacity) contained the following components (in μmol.): *tris* (hydroxymethyl) aminomethane buffer, *p*H 7·5, 80; sodium ascorbate, *p*H 7·5, 10; manganese chloride, 2; chloroplasts (5–10 mgm. chlorophyll) suspended in sodium chloride, 1,540. In Exps. 1 and 2, the mixture contained $NaH^{13}CO_3$, 48 and 5 μmol. respectively. In Exp. 3 the carbon-12 dioxide was negligible. The total volume was 4·8 ml.

The side-arm carried 1 μmol. of carbon-14 dioxide as sodium bicarbonate containing in μc.: Exp. 1, 100; Exp. 2, 150; Exp. 3, 10. After the vessels were flushed with prepurified nitrogen in a bath at 14° C. for 10 min. with the lights on (4,000 foot-candles), the isotopically labelled sodium bicarbonate was tipped in. The light source was Sylvania Birdseye Floodlites (150 watts, 120 volts). The reaction was terminated by heating the cup contents in a boiling water bath for 2 min. After the residue was washed twice in the centrifuge tube with hot water, the starch glucose was derived by hydrolysis with 6 ml. of 1 *N* hydrochloric acid for 45 min. at 100°. The mixture was centrifuged and the residue was washed twice with hot water. The supernatant fluid and washings, brought to approximately *p*H 7·5, were passed through 'Dowex-50' (H^+ form) and 'Amberlite *CG*-45' (OH^- form). The effluent was evaporated to approximately 1 ml. and streaked on to washed sheets (14 cm. × 17 cm.) of Whatman No. 1 paper. The chromatograms were developed with butanol (52 ml.), 95 per cent ethanol (32 ml.) and water (16 ml.). The X-ray film showed only one band with an R_F value corresponding to glucose. This band coincided with the typical hexose colour developed after aniline phthalate spraying. This glucose, together with 400 μmol. of carrier glucose, was degraded with *Leuconostoc mesenteroides*[3]. Activity is expressed in Table 1 as mμc. per mgm. of carbon.

The ratio of carbon atom 3 to carbon atom 1 (C-1, aldehyde carbon) and/or C-2 is less than that of C-4 to C-5 and/or C-6. This type of labelling pattern is similar to that reported by Gibbs and Kandler for glucose formed in intact cells[1]. It would appear that the cause of asymmetry is probably not a relatively large supply of unlabelled dihydroxyacetone phosphate.

The similarity between the tracer pattern of the starch glucose formed by the intact cells and isolated chloroplasts further strengthens the concept that the isolated structure possesses a typical photosynthetic carbon cycle.

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Direct Absorption of Dissolved Strontium-90 and Yttrium-90 by Tadpoles of *Rana temporaria*

The presence of long-lived radioisotopes, for example strontium-90 and cæsium-137, in fall-out material and their detection in soil, vegetation and animal products have been confirmed[1]. The harmful effects of this build-up of radioisotopes can, to some extent, be predicted by reference to earlier experiences with radioactive materials such as radium. There is very little experimental evidence on the direct biological effects of strontium-90 and cæsium-137. A long-term investigation into the life-cycle of the frog has been started and some exploratory results of the turnover of radiostrontium and radioyttrium by the tadpoles of *Rana temporaria* are of interest.

Tadpoles, in batches of 100, from local ponds, estimated to be 5–7 weeks old, were treated with small amounts of strontium-90, about 0·125 μc. in 500 ml. water containing 100 tadpoles, giving $2·5 \times 10^5$ disintegrations/min. strontium-90 and yttrium-90. The experiments were conducted in glass tanks; the exchange of activity by the glass was shown to be small. The water was tested for radioactivity at intervals. The tadpoles were eventually removed from the water, which was filtered through a sintered crucible to collect the insoluble matter, mainly fæces. The fæces were dissolved in hot nitric acid, which was then suitably diluted. A sample of 10 tadpoles was washed, incinerated, extracted with aqua regia and suitably diluted. The tank contents were, therefore, divided into: (1) water; (2) fæces solution; (3) tadpole solution.

The activities of all three solutions were measured at further intervals of time using a type *M*6 Geiger–Müller liquid counter and associated *IDL* counting equipment (scaler, probe unit, and extra-high tension unit). The performance of the counting equipment was checked throughout by means of a standard uranium solution. The background was also checked and found to be practically constant at 13 c.p.m. Five

Table 1

Sample	Strontium-90 (c.p.m.)		Yttrium-90 (c.p.m.)		Strontium-90/yttrium-90 at 175 hr.	
	0 hr.	175 hr.	0 hr.	175 hr.	From liquid counts	From solid source absorption plot
Water	34,000 (*A*)	28,000 (*C*)	34,000 (*A*)	9,500 (*B*)	3·0	3·1
Tadpoles	—	6,000 (*E*)	—	19,300 (*D*)	0·31	0·32
Excreta	—	1,500 (*G*)	—	4,000 (*F*)	0·38	—
Totals	34,000	35,500	34,000	32,800	—	—

experiments as above have been carried out to date, and a typical set of results is represented in Fig. 1.

The decay scheme is as follows:

$$^{90}\mathrm{Sr} \xrightarrow[\substack{0\cdot61\ \mathrm{MeV.}\\ 28\ \mathrm{years}}]{\beta} {}^{90}\mathrm{Y} \xrightarrow[\substack{2\cdot25\ \mathrm{MeV.}\\ 64\ \mathrm{hr.}}]{\beta} {}^{90}\mathrm{Zr}$$

It is apparent that the liquid counter is measuring mainly the 2·25 MeV. β-emission of yttrium-90 and very little of the strontium-90 0·61 MeV. β-emission. The glass wall thickness is about 35 mgm./cm.[2] but the effective absolute thickness may be several times this value[2]. The initial drop in curve *AB* is due mainly to absorption of yttrium with some strontium by the tadpoles. The levelling out of *AB* is probably due to the absorption of yttrium as soon as it is formed from the strontium remaining in the solution. After separation of the tadpoles and fæces, the count on the water rises along curve *BC*. This indicates the growth of yttrium in the water from the strontium remaining, until a fresh secular equilibrium is reached at *C*. This value at *C* represents the yttrium now in equilibrium with the strontium not removed by the tadpoles. The counts on the tadpole solution *DE* and the fæces solution *FG* decrease with passage of time to constant levels at *E* and *G*.

Points *C*, *E* and *G*, therefore, represent the amounts of strontium-90 in the water, tadpoles and the fæces respectively at the time of separation, that is, 175 hr. Points *D*, *B* and *F* represent mainly the yttrium-90 content of the three solutions at separation.

Thus, the tadpoles have a strontium-90/yttrium-90 ratio at separation of 6,000/19,000 or 1/3·1. An absorption plot was carried out on an aliquot of the tadpole solution evaporated to dryness on a planchette and almost the same strontium/yttrium ratio was obtained. All the results are summarized in Table 1.

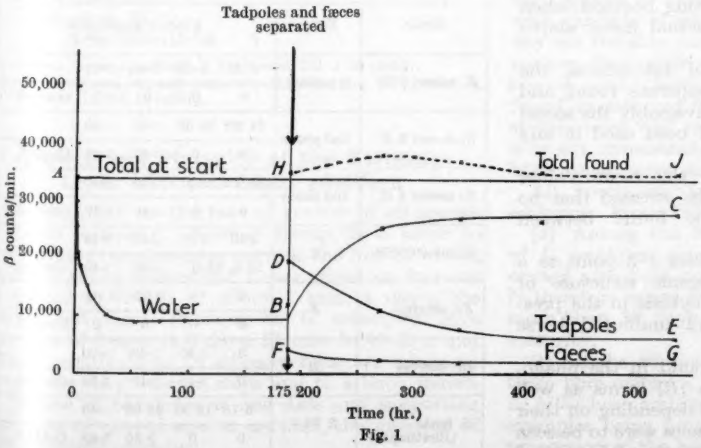

Fig. 1

The 100 tadpoles absorbed in about one week approximately 18 per cent (~0·02 μc.) of the strontium-90 and about 72 per cent (~0·09 μc.) of the yttrium-90.

Attempts were made to wash the strontium-90 from the tadpoles with water, but, even after four weeks, the tadpoles still had a strontium-90 content not appreciably lower than indicated by point *E* (Fig. 1). Ethylenediamine tetraacetic acid (0·01 per cent) solution increased the rate of decontamination. Preliminary investigations indicate that calcium bicarbonate added to the tank results in a lower uptake of strontium-90 by the tadpoles, although the curves obtained are similar to those for soft water. Histological changes and the effect on metamorphosis are being investigated.

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Phagocytosis and the Antigenic Structure of Bacteria

Phagocytosis is one of the mechanisms underlying immunity against infection. In spite of numerous investigations, our knowledge concerning this mechanism is still inadequate. No systematic studies have been conducted on the significance of the antigenic structure of bacteria in the process of phagocytosis, and the data on substances contained in the blood serum promoting phagocytosis and referred to as opsonins or tropins are also inadequate.

The present experiments aimed at elucidating the importance of antigenic structure of bacteria in the phagocytosis process in the case of Gram-negative intestinal bacilli with known antigenic structures.

Horse sera from healthy, non-immunized horses were used in all experiments. These sera were inactivated on the day of experiment for 30 min. at a temperature of 56° C. in order to exclude the participation of the complement, and were used in an undiluted state.

Leucocytes from horse blood were obtained by centrifugation of mechanically defibrinated blood; they were washed five or six times by centrifuging

Table 1. Phagocytosis of Bacteria with *K* Antigens

Antigen	Strain	Heat susceptibility of antigen	Kind of suspension *f* 60° C.	100° C.	120° C.		Control
Vi	*S. typhi* *S*–6 *Vi*+	60° C.	0	0	4·0	>40	0
	O 901 *Vi*−		1·55	13·75	>40	>40	0
M	*S. paratyphi B*	100° C.	9·51	1·71	>40	>40	0
L	Alkalescens–Dispar *O*1 *L*+	60–100° C.	0	22·96	>40	>40	0
	L−		>40	>40	>40	>40	0
B	*S. dysenteriae* 2 (*S. schmitzii*)	100° C.	0·54	0·80	>40	>40	0
A	Alkalescens–Dispar *O*6 *A*+	120° C.	0·55	1·60	7·65	>40	0
	A−		5·32	>40	>40	>40	0

with physiological saline at the speed of 2,000 r.p.m. The leucocytes were used for experiments 3–4 hr. after the blood had been taken.

The strains used for experiments were : *Salmonella*, *Shigella*, *Alkalescens-Dispar*, *Escherichia* and *Brucella*. The bacterial suspensions were washed with physiological saline to remove any soluble antigens which might affect the results of the phagocytic reaction. The suspensions to be examined were checked for the presence or lack of the *K*, *O* and *R* antigens.

The phagocytic reaction was performed by the tube method ; one volume of washed leucocytes was mixed with one volume of inactivated undiluted serum and with one volume of the suspension of the bacterial strain under examination. After rough mixing, the tubes were placed in a water-bath at 37° C. for 20 min. (In control tests physiological saline was used instead of horse serum ; the time of phagocytosis was prolonged to 60 min.) After drying in air, the preparations were fixed by passing them three times over a flame and then for 20 min. in methyl alcohol. The preparations were then stained with undiluted Manson dye by immersion for 3–5 sec. ; afterwards they were thoroughly washed and dried in air.

Counts were made using an oil-immersion objective. The phagocytic index was calculated by dividing the number of bacteria digested by 100 leucocytes by 100. In view of the difficulty of counting bacteria when their number exceeded 40, the actual index above 40 was assumed, not calculated.

To minimize the possibility of side-effects, the suspensions were prepared in a separate room, and the persons reading the results (invariably the same) did not know what bacteria had been used in any given investigation.

The tabulated results present the mean values from six experiments. It is to be stressed that no pronounced differences could be found between particular sera.

The results summarized in Tables 1–3 point to a close relation between the antigenic structure of bacteria and the degree of phagocytosis in the presence of normal, inactivated and undiluted horse serum.

Pronounced differences were found in the phagocytosis of smooth (*S*) and rough (*R*) forms as well as in the phagocytosis of *S*-forms depending on their antigenic structure. These differences were to be seen both with the use of naturally dissociated forms and with suspensions in which some antigens had been destroyed by heating at temperatures of 60°–120° C.

It is of interest that there is complete agreement between the results obtained in the phagocytic reaction and those by serological methods, used as a matter of routine in the experiments on the antigenic structure (agglutination, absorption tests).

The results contained in Table 1 point to the great importance of the surface antigens (*Vi*, *M*, *L*, *B*, *A*) for the phagocytosis of bacteria which possess them. *K* antigens exert a pronounced inhibitory effect on phagocytosis. A clear-cut relation could be established between heat susceptibility and the extent of phagocytosis.

Table 2. Phagocytosis of Bacteria without *K* Antigens

Antigen	Strain	Heat susceptibility of antigen	Kind of suspension *f* 60° C.	100° C.	120° C.		Control
—	*S. flexneri X*	—	4·61	>40	>40	>40	0
—	*S. flexneri Y*	—	10·20	>40	>40	>40	0
I	*S. flexneri* 1*b*	—	0·78	1·83	>40	>40	0
III	*S. flexneri* 3	—	0·46	4·38	>40	>40	0
alpha	*DRL* 358/55	100° C.	0	1·90	>40	>40	0
beta	*E. coli* C_3	100° C.	1·92	4·32	>40	>40	0
1*F*	*S. flexneri* 6	120° C.	0	2·75	37·50	>40	0

The results in Table 2 suggest the possibility of differentiating the bacteria in which no *K* antigen could be found (*O* forms). Here, too, differences in the degree of phagocytosis were observed depending on the heat susceptibility and the character of antigen (the presence of thermolabile α-, β-, 1*F* antigens ; the presence or lack of type antigens).

The results in Table 3 suggest the possibility of differentiation between the smooth (*S*) and rough (*R*) form and the detection of the transitional *SR* forms. It was found that *R* forms are also phagocytized in the absence of serum ; this fact distinguishes them from *S* forms, which undergo no phagocytosis in these conditions.

The results presented indicate the great importance of the structure of the bacterial cell in the phagocytic process. They also suggest new possibilities of studies

Table 3. Phagocytosis of Smooth (*S*), Rough (*R*) and Transitional *SR* Forms

Strain	Form	Kind of suspension *f* 60° C.	100° C.	120° C.		
S. sonnei 9773	1st phase	0·93	1·80	3·80	>40	
		0	0	0	0	Control
S. sonnei 3 *H*	2nd phase	21·20	30·50	>40	>40	
		0	0	>40	>40	Control
S. sonnei 4 *H*	2nd phase	28·16	>40	>40	>40	
		0	1·0	>40	>40	Control
S. sonnei 9774	*R*	>40	>40	>40	>40	
		15·0	25·0	>40	>40	Control
Br. abortus	*S*	0	0	2·55	3·03	
		0	0	0	0	Control
Br. abortus	*R*	0	>40	>40	>40	
		0	>40	>40	>40	Control
S. typhi (Bhatnagar)	*R Vi*+	6·18	15·90	28·03	>40	
		0	0	5·60	>40	Control

on the antigenic structure of bacteria by the application of the phagocytic reaction.

The experiments shed some light on the efficiency of the mechanism of phagocytosis depending on the character of infecting bacteria. They also suggest the importance of an as yet unknown factor, contained in the normal horse serum, for the phagocytic process of smooth bacterial forms.

The phagocytic test proved to be more sensitive than serological reactions in the detection of differences in the antigenic structure of bacteria (for example, in the detection of transitional forms).

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Longevity of *Proteus* Group of Amœbæ

Explanation (1) of the findings described by Muggleton and Danielli[1] appears to fit in best with the facts enumerated below.

The normal life-cycle of the *Proteus* group of amoebae consists of four well marked stages: (*a*) spore, (*b*) developing juvenile, (*c*) adolescent, (*d*) mature to senile. It is the adolescent and young adult that can be made to reproduce indefinitely by binary fission. As soon, however, as a rigid control of their environment is relaxed, they proceed with their life-cycle. Chromidia from the nucleus escape into the cytoplasm and develop into spores. When the individual becomes filled with spores it dies. If, however, these spore-filled individuals are placed in a Petri dish and killed by a change in *p*H, the hatching of the spores, and their subsequent development, given a suitable pabulum, can be observed. It has been shown why this development is slow[2]. I am at present engaged in trying to accelerate this growth, and am glad to record here the gift of supplies of bacteria and lettuce-water sent to me by Dr. Muriel Robertson of the Lister Institute.

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Growth and Maturity of the Barnacles *Lepas hillii* and *Lepas anatifera*

Information on the rate of growth of all species of *Lepas* is scanty. Stanley Kemp, in a letter to J. F. Anton[1], quotes three sources, the first of which, referring to an unspecified *Lepas*, gives an increase in capitulum length of about 1 mm. a day; the second and third, referring to *L. anserifera*, give increases of 8 mm. in 8 days, 21 mm. in 40 days and 25 mm. in 107 days. In all cases these are minimum rates since they refer to ships and to a buoy known to be free of barnacles at one date and supporting barnacles of quoted size at another, the moment of settling being unknown.

During the voyage of the yacht *Petula* from Dakar to Barbados in the winter of 1953–54 [2], colonies of barnacles settled on the ship's side above her anti-fouling paint and flourished there throughout the passage. While the ship lay in Dakar harbour preparing for sea, the stores which were loaded into her so increased her draught that some of her white topside paint was submerged, and here many fouling organisms were able to gain a hold. So long as the ship was at anchor only the sessile barnacle *Balanus amphitrite* Darw. was present, but at sea this species largely died off and was replaced by the pedunculate barnacles *Lepas hillii* Leach, *Lepas anatifera* L. and *Conchoderma virgatum* (Spengler). *Conchoderma virgatum* was present in large numbers and early attained a capitulum length of 9 mm. but beyond this length it did not grow, being heavily preyed upon by the trigger fish *Canthidermis maculatus* and *C. sobaco*.

In position 15° N., 31° W., after the ship had been at sea for 30 days, a sample of twenty of the largest *Lepas* growing on the topside was taken and preserved. A further sample of sixty of the largest specimens from an adjacent position was taken after 60 days, in position 15° N., 46° W.

Measurement of the preserved material yielded results summarized in Table 1. The measurements, to the nearest 0·5 mm., were made from the umbo (basioccludent angle) of the scutum to the upper (occludent) tip of the tergum. This dimension may be approximately equated with the capitulum length generally quoted in the literature, being very slightly greater; it is to be preferred to the capitulum length as giving a firmer standard of size.

Table 1. Length Distribution of *Lepas* taken 30 and 60 Days Out

	Length (mm.)	11–13·5	14–16·5	17–19·5	20–23
30 days	Total *L. hillii*	3	4	2	0
	Gravid *L. hillii*	0	1	0	0
	Total *L. anatifera*	5	6	0	0
	Gravid *L. anatifera*	0	2	0	0
60 days	Total *L. hillii*	6	10	20	21
	Gravid *L. hillii*	0	2	16	11
	Total *L. anatifera*	0	0	1	2
	Gravid *L. anatifera*	0	0	0	1

The age of the barnacles in the samples is again not known with certainty since settlement on the ship's side was not observed. Colonies containing both species of *Lepas* were established 10 days out, with capitula of perhaps 5 mm., and from the first day out the ship passed among much flotsam bearing mature *L. anatifera* from which infection by this species could have occurred. No *L. hillii* were found on collected flotsam, yet it is certain that both species were well established on the tenth day and it is not unreasonable to suppose that settlement of both began very near to the start of the voyage.

All specimens were opened and searched for embryos. From Table 1 the following may be noted.

(*a*) Among the 30-day group only one specimen of *L. anatifera* among eleven and one of *L. hillii* among nine bore embryos in the ovigerous lamellæ. It therefore seems probable that both species require more than 30 days on average from settlement to maturity.

(*b*) Of the 60-day group of *L. antifera* one out of three bore embryos. Twenty-nine out of fifty-seven *L. hillii* bore embryos, all except two of the gravid examples being longer than 16·5 mm. So large a proportion of the barnacles between 17 and 19·5 cm.

contained embryos that it is probable that this span represents first maturity. Since *L. hilli* achieved, in general, a length of 13–17 mm. in 30 days, 17–19·5 mm. at maturity and 17–21 mm. in 60 days it might be crudely assumed, given a constant increase in length during the second month, that maturity was achieved, on average, in the last two weeks of the second month. Comparing lengths at 30 and 60 days, however, it is seen that the average daily increase of about 0·5 mm. in the first month is not maintained in the second. This check in growth was no doubt due to the attainment of maturity at 17–19·5 mm. It would therefore seem more probable that the growth-rate of 0·5 mm. a day observed in the first month continued to maturity, which on these terms occurred in general on the thirtieth to forty-third day. Thereafter, a growth-rate near 0·03 mm. a day is indicated.

Throughout the voyage the sea temperature was high, ranging from 24·2° to 26·1° C. The conclusions advanced above concerning both growth-rate and the length of the juvenile phase must consequently be applied with caution to animals taken in cooler waters.

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Occurrence of Forms of *Fucus distichus* L. emend. Powell on North Rona and Sula Sgeir

Fucus distichus L. emend. Powell is an extremely polymorphic alga, widely distributed principally on arctic and sub-arctic coasts, and I have recently proposed[1] the recognition of four principal subspecies of this plant. Two of the subspecies—subsp. *edentatus* (De la Pyl.) Powell (a large plant of sheltered shores), and subsp. *anceps* (Harv. et Ward ex Carruthers) Powell (a small plant of exposed shores)—have southern limits in northern and western parts of Scotland and Ireland[2], and are the rarest fucoids in Britain.

As a member of the Glasgow University North Rona Expedition (June 23–July 23, 1958)[3], I have recently been able to investigate the botany and littoral ecology of the small, remote Scottish islands of North Rona and Sula Sgeir which lie about 45 miles north-west of Cape Wrath. Subsp. *anceps* was found on both islands, in small quantity but at several sites in each case, at its usual high level on shores exposed to severe swell and wave action. Subsp. *edentatus* was found on North Rona only, in the moderately sheltered inner parts of two narrow geos facing north and north-east respectively, in the north-eastern part of the peninsula of Fianuis. In the north-facing geo, only a few young (non-fertile) plants were found on large mid-littoral boulders; but in the north-east-facing geo, subsp. *edentatus* formed a distinct belt (of varying depth) in the upper half of the mid-littoral zone, along about 25 m. of shore each side of the geo, and both young (non-fertile) and older (fully fertile) plants were present. The largest plant was 42 cm. in length. Several plants of both subspecies have been examined in detail; caecostomata[1] were present in all, and the conceptacles were hermaphrodite. The only other fucoid found was *F. spiralis* L. (mostly f. *nana*)—very scarce on North Rona only.

The present findings confirm previous observations[2] that subsp. *anceps* can survive more severe exposure conditions than any other fucoid in northern Britain; and these records from such small and isolated islands strengthen my belief[2] that subsp. *anceps* is a relict plant in Britain, a survivor from a period when the climate here was colder.

Subsp. *edentatus* has been reported previously from only three localities in Britain (in the harbours of Lerwick and Scalloway in the Shetland Isles, and in the North Haven on Fair Isle)[2]; the North Rona records therefore establish a new southern limit for the plant on the British side of the North Sea. I have suggested elsewhere[2] that subsp. *edentatus* may have been introduced to the Shetland and Fair Isle localities within recent centuries, perhaps by fishing boats; but it seems scarcely likely that it could have been introduced to North Rona in this way, even though we saw as many as four Norwegian fishing boats at a time anchored close inshore there during periods of rough weather. The possibility of introduction by floating plants cannot be ruled out, of course, but this seems not very likely in view of the prevailing winds and currents. There remains the possibility that subsp. *edentatus* has been present on North Rona, and perhaps at the other three Scottish localities, a very long time—a relict plant, as subsp. *anceps* appears to be; I now much incline to this view.

A detailed report on the littoral flora and fauna of North Rona and Sula Sgeir is being written in collaboration with my colleague Mr. T. B. Bagenal, and will be published elsewhere.

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Selection of Cell Lines in Tissue Culture by the Use of Cytotoxic Antisera

The cytotoxic action of antisera against nucleoproteins isolated from HeLa, H.Ep. No. 1 and MCN cells has been previously reported from this laboratory[1,2]. Of particular significance was the observation that only cell lines exhibiting epithelial-like morphology (Ep. L) were destroyed by the HeLa and H.Ep. No. 1 antisera, while cells with fibroblast-like morphology (Fb. L) were affected only by the MCN antisera. Consistently, cultures of susceptible cells were completely destroyed by a 1 : 50 dilution of cytotoxic antisera in the presence of guinea pig complement. During the course of these investigations, an established strain of human amnion cells was encountered the behaviour of which toward HeLa and MCN antisera was different from that of cell lines previously examined. A fraction of the cell population was resistant to the MCN antiserum and a fraction was resistant to the HeLa antiserum, even when the antisera were employed at 1 : 4 dilution. It is the purpose of this communication to report the selection of two cell lines from the human amnion strain. These two sublines can be distinguished on the basis of their morphological characteristics, their capacity to support virus multiplication, and their sensitivity to the HeLa and MCN antisera.

The human amnion strain was obtained through the kindness of Dr. L. Hayflick from the Tissue Culture Laboratory of the University of Texas,

Galveston, Texas. The establishment and development of this cell strain have been described recently by Fernandes[3]. From its initial isolation, this strain has exhibited epithelial-like characteristics. It has not undergone any of the changes often associated with the development of an established cell line from a primary culture[4]. The cell supported with evident cytopathogenic changes the growth of a number of viruses, including types I, II and III poliomyelitis[3].

The human amnion strain was received in this laboratory on January 21, 1958. It was observed that the cells grew vigorously, although their general growth pattern was not typical of that associated with epithelial-like elements. The cells were mainly of the fusiform type. During initial experiments with poliomyelitis virus, no obvious cytopathology could be ascertained. The cells were tested for their susceptibility to the HeLa and MCN nucleoprotein antisera by the method described[1,2]. Neither antiserum, even at low dilutions, was capable of destroying all the cells. The HeLa antiserum destroyed a maximum of 10–15 per cent of the cell population while the MCN antiserum destroyed at most 85–90 per cent of the cells. Identical results were obtained with undiluted antisera and with antisera diluted 1 : 10, indicating that over this range the percentage of cells destroyed by the antisera was not a function of the concentration of cytotoxic antibodies. Attempts were then made to isolate the two types of cells by means of selective destruction by the two antisera. The procedure followed is described below.

Cells were grown on glass (prescription bottles, 8 oz.) in a medium consisting of 10 per cent horse serum in Eagle's basal medium[5]. They were scraped from the glass wall and suspended in growth medium to give a concentration of 10^6 cells/ml. 2 or 3 ml. aliquots of this suspension were incubated with HeLa or MCN antisera (final dilution 1 : 4) in the presence of guinea pig complement. After 2 hr. the percentage of surviving cells was recorded, the cell suspensions were centrifuged, and the sedimented cells were planted in separate bottles in 90 per cent Eagle's medium and 10 per cent horse serum.

In a successful experiment, growth was observed after treatment with the antisera. Cells that had survived the MCN antiserum treatment grew as separate colonies, each cell having polyhedral contours and large areas of contact with its neighbours. These characteristics were recognizable as epithelial (Ep. L). In contrast, those cells that had survived HeLa antiserum treatment were mostly fusiform with filamentous processes and had no tendency to form separate colonies. This morphology and growth pattern are associated with fibroblasts (Fb. L). Cells of the first type (Ep. L) were completely destroyed by the HeLa antiserum while MCN antiserum completely destroyed the second (Fb. L) type. The two sublines were then transferred and separately maintained. The isolated human amnion sublines were tested for sensitivity to the three types of poliomyelitis virus. The virus pools employed were cultures of monkey kidney cells infected with the virus agents. The results of titrating these three pools in tubes of the amnion Ep. L and Fb. L lines, as well as the initial titre in the monkey kidney cultures are shown in Table 1. All three types of polio produced cytopathogenic changes in the Ep. L line through 10^{-5}–10^{-6} dilutions of virus, while no cytopathology was observed in the Fb. L line even at the highest virus concentrations. Production of type II polio virus by the two sublines was studied by back titration of infected culture fluids on monkey kidney cells. Ep. L cells were exposed to a 10^{-5} dilution of type II virus; the medium was harvested and titrated on monkey kidney cells through five serial ten-fold dilutions. A five-log increase in virus concentration was demonstrated in the Ep. L cells. By contrast, when culture fluids from the Fb. L cells were titrated in monkey kidney cultures, no increase in virus concentration could be demonstrated. Several passages of type II polio in the Fb. L cells yielded no virus as could be determined by back titration in monkey kidney.

During four months of observation, the Fb. L line has remained unchanged, while in the Ep. L line there has been a constant increase in the number of cells sensitive to the MCN antiserum. This tendency has been controlled by periodic treatment of the cells with MCN nucleoprotein antiserum until a stable epithelial line was obtained. In some bottles which have not received this treatment, the MCN antiserum-sensitive cells have increased after four transfers to the level observed in the original culture (85–90 per

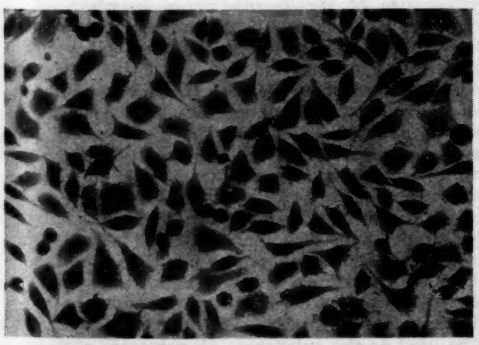

Fig. 1. Human amnion, Fb. L subline. Hæmatoxylin and eosin. (× 160)

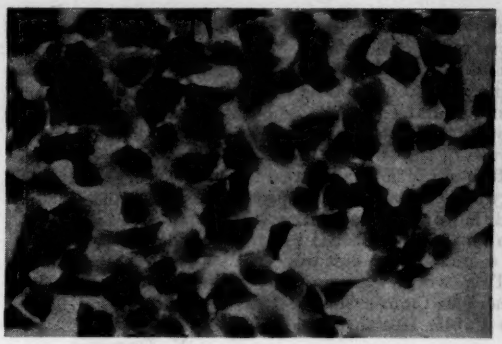

Fig. 2. Human amnion, Ep. L subline. Hæmatoxylin and eosin. (× 160)

Table 1. Titration of types I, II and III Poliomyelitis Virus in Monkey Kidney, Amnion Ep. L and Amnion Fb. L Cells

Virus	*TCD*50/ml.*		
	Monkey kidney	Amnion Ep. L	Amnion Fb. L
Type I, Mahoney	7·0	5·4	†
Type II, MEF,	6·5	5·0	†
Type III, Saukett	6·7	5·8	†

* Expressed as logarithms to the base 10.
† No cytopathology seen at any virus concentration.

cent). This phenomenon probably represents the more rapid proliferation of some fibroblast elements left among the epithelial cells, a possibility which is being studied by cloning techniques. In addition, the number and morphology of the chromosomes, the growth characteristics, and certain biochemical properties of the two cell lines are under investigation.

It is of particular interest that the cellular morphology is significant with respect to virus susceptibility[6,7] and that a cell type with certain specific characteristics can be rapidly selected by the application of antisera to a mixed cell culture.

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Antigenicity and Survival of Cartilage Homografts

During the course of investigation of the reasons underlying the ability of cartilage grafts to survive in foreign hosts for long periods, the effects of cartilage homografts and heterografts on the regional lymph node were studied in order to determine whether such grafts were antigenic[1]. The results of this test indicated that both types of graft do evoke an active immune response in the host animal, and it was discovered, further, that heterografts do so even after having been boiled for periods of up to 10 min.[2]. This test does not, however, indicate whether the host animal becomes fully immunized by the foreign cartilage, and in order to determine whether this is so, rabbits bearing cartilage homografts have been challenged with skin homografts from the cartilage donor.

Thirty-six rabbits were used in the investigation. Eighteen control rabbits received a fitted skin homograft of 1½ in. × ½ in. Eighteen experimental animals were given 0·2 gm. of diced homologous cartilage on each of two occasions four days apart, followed by a fitted 1½ in. × ½ in. skin homograft from the cartilage donor on the fourteenth day after the second cartilage implant. A skin homograft of 0·36–0·44 gm. weight will fully immunize an adult rabbit within 14 days[3]. All 36 skin homografts were removed on the sixth day after grafting, and 7μ paraffin sections were stained by hæmatoxylin and eosin. The graft condition was assessed histologically, using the following classification: (*A*) survival, with proliferation, of the entire graft epithelium; (*B*) breakdown of proliferated or still proliferating epithelium in progress; (*C*) breakdown of non-proliferated graft epithelium in progress or complete. 'Immune' type of breakdown.

The results are presented in Table 1. They indicate that, in the majority of cases, implantation of 0·2 gm. of homologous cartilage from the same donor on two occasions is insufficient to elicit a state of transplantation immunity in the host. Only five (28 per cent) of 18 rabbits so grafted appear to have become fully immune.

Table 1

Graft condition	Control animals	Experimental animals
A	8	4
B	10	9
C	0	5

This finding raises the question of whether cartilage homografts survive because of their mild degree of antigenicity. This does not appear to be the case, since further experiments I have carried out show that cartilage homografts can survive in animals fully immunized by skin homografts from the same donor. These findings will be published in greater detail elsewhere. The ability of cartilage homografts to survive may therefore well be attributable to the peculiar physico-chemical character of the matrix, as was first postulated by Bacsich and Wyburn[4]. The matrix would appear to behave after the fashion of the semi-permeable diffusion chamber devised by Algire, Weaver and Prehn[5], which allows foreign cells to survive in an immune host.

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A Technique for the Investigation of the Competitive Saprophytic Ability of Soil Fungi by the Use of Easily Decomposed Substrates

The method commonly used to determine the competitive saprophytic ability of soil fungi consists of exposing suitable substrates to colonization by the fungus in non-sterile soil. A serious disadvantage of this method is that only relatively resistant substrates, for example, wheat straw, can be used effectively because of the manipulation to which the substrate has to be subjected after incubation. Less-resistant substrates such as portions of non-woody roots, rhizomes and cylinders or disks of storage parenchyma of carrots or potatoes, etc., are so softened by the action of soft-rotting organisms in the soil that it becomes difficult to cleanse them adequately of adhering soil, and hence to state with confidence that fungi isolated from them have actually colonized the substrate in competition with the general soil microflora. It is very important to test the suitability of any potential substrate, for, as Garrett[1] has pointed out in the case of root-infecting fungi, one is usually trying to demonstrate a negative character, that is, inability to compete as a soil saprophyte, and to obtain the strongest circumstantial evidence the killed host must be used as the substrate. A substrate such as wheat straw can probably only yield evidence relevant to fungi pathogenic to wheat or at most to cereals;

hence the need to be able to employ any substrate that suggests itself. Since the chief obstacle to the use of soft tissues as substrates is the difficulty of removing the unwanted soil, the present method has been designed to prevent all unnecessary contact with the soil.

The rhizome of *Convolvulus sepium* was the substrate used in the development of this technique. Lengths of rhizome were washed free from soil, blotted dry, wiped with a pad of cotton-wool soaked in 95 per cent alcohol and washed again in tap water. When dry the pieces of rhizome were placed inside 1¼ in. lengths of sterilized glass tube of suitable diameter and trimmed until approximately ⅛ in. of tissue protruded from each end of the tube. A number of tubed substrates were exposed to propylene oxide vapour in a sealed container for 44 hr. Rhizome thus treated yielded no fungi when plated on potato dextrose agar. The sterile tubed substrate was then exposed to colonization by fungi in various soil/inoculum mixtures maintained at a pre-determined moisture content. At the end of the incubation period batches of ten tubed substrates, which had been placed horizontally in the soil, were recovered and treated as follows: The protruding ends were trimmed off and the tubes placed in a 250-ml. beaker. They were washed in a strong jet of water, using about 500 ml. of water in all. The rhizome segments were then removed from the tubes, washed in two changes of tap water, shaken in 50 ml. of 0·1 per cent mercuric chloride in 10 per cent alcohol for 1 min. and finally washed in three 100-ml. portions of sterile streptomycin sulphate solution (51·8 I.U./ml.). The washed substrates were then plated on potato dextrose agar and incubated at 25° C. for 10 days.

Table 1 illustrates the use that is being made of this technique in a study of the ecology of some members of the genus *Cylindrocarpon* Wollenweber. The results were obtained by examining the incubated substrates and the surrounding medium for the characteristic macroconidia of these fungi. An arbitrary method of scoring was used, 10 units being given to each rhizome segment which yielded macroconidia. Some species of *Cylindrocarpon* tend to produce mostly microconidia which are less characteristic of the genus than the macroconidia. Segments bearing only microconidia were therefore disregarded, even though it was extremely likely that the test fungus had colonized the segment. *Fusarium culmorum* was included in the tests for comparative purposes since several workers have shown that this organism possesses a high degree of competitive saprophytic ability.

These results indicate that this technique is sufficiently sensitive to distinguish between the competitive saprophytic ability of members of the same genus. The technique can be adapted in various ways. For example, by cutting the substrate into 2 mm. sections and plating these in known sequence on agar, information can be obtained not only about the ability of a fungus to colonize the substrate in competition with the general soil micro-flora but also about its rate of spread along the substrate.

Table 1. PERCENTAGE COLONIZATION OF RHIZOME SEGMENTS OF *Convolvulus sepium* BY *Cylindrocarpon* spp. AND *Fusarium culmorum* IN A RANGE OF INOCULUM/SOIL MIXTURES

Percentage inoculum/soil	0	2	10	50	90	98	100
F. culmorum	100	100	100	100	100	100	100
C. chrenbergii	0	0	0	20	100	80	100
C. olidum	0	0	10	30	80	80	100
C. janthothele var. *minor*	0	0	0	0	10	10	100
C. candidum	0	0	0	0	0	10	100
C. obtusisporum	0	0	0	0	0	20	100
C. radicicola	0	10	0	0	10	20	100
C. sp. (T.W.3)	0	0	0	0	0	10	100

We wish to thank Dr. W. L. Gordon of the Plant Pathology Laboratory, Winnipeg, who supplied some of the isolates of *Cylindrocarpon*.

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A Direct Method for Determination of Forward-Mutation Rates in *Neurospora crassa*

THE main difficulty in estimating forward-mutation (from wild type to mutant) rates at specific loci in micro-organisms is the determination of the total number of specific mutations in a given population of cells after mutagenic treatment. In general, mutation rates are so low that the number of specific mutants cannot be readily determined by direct methods. An attempt has been made to determine forward-mutation rates at specific loci[1] by indirect methods, using the 'filtration-concentration' technique, which permits only partial recovery of the induced mutants. Later, an effort was made to determine what proportion of the total mutants induced at each of these loci is recovered with such techniques, by reconstruction experiments in which various mixtures of wild-type and mutant conidia were used. To circumvent the various assumptions that must be made to obtain estimates of the forward-mutation rates from the data obtained by such indirect methods, we developed an experimental technique that permits the estimation of the forward-mutation rates of specific loci by a direct method.

The colonial microconidiating albino (*al*-2) strain, 76–*OR2–7A*, gives white spherical colonies with very restricted growth-rates either on solid media in Petri plates or in viscous media in liquid culture. Success of the new method depends on morphological distinction between certain mutants and wild type in such a genetic background. In *ad*-3 mutants, a purple pigment is accumulated in the mycelium that readily distinguishes such mutants from wild type. Moreover, production of this pigment can be enhanced by use of growth-rate-limiting levels of adenine and by incubation at 37° C. Thus intermediate alleles with only partial requirements for adenine can also be recovered. After mutagenic treatment of conidial suspensions of strain 76–*OR2–7A*, aliquots were inoculated into 20-l. bottles filled with 12–15 l. of minimal medium supplemented with 1 μgm. of adenine sulphate per ml. and made viscous by the addition of 0·15 per cent agar. In this medium, individual conidia are kept well separated and, instead of clumping together, develop into distinct spherical colonies. The medium is stirred by slow aeration through a 6-mm. glass tubing constricted at the tip. All bottles were incubated at 37° C. When individual colonies had developed into spheres 2–3 mm. in diameter, the contents of each bottle were poured into white enamelled developing trays (40 cm. × 60 cm.) to form thin layers. The purple adenine mutants were recovered on the basis of the characteristic purple

Table 1. The Frequency of X-Ray Induced Forward Mutations at the *ad*-3*A* and *ad*-3*B* Loci

Dose (kr.)	Survival		Total purple mutants	*ad*-3*A* mutants			*ad*-3*B* mutants		
	No. of conidia analysed $\times 10^6$	Percentage of initial		No. found	Mutants per 10^6 viable conidia		No. found	Mutants per 10^6 viable conidia	
					Initial	Surviving		Initial	Surviving
None	7·60	100·0	0	0	—	—	0	—	—
4·5	6·64	87·5	16	3	0·39	0·45	13	1·7	2·0
18	5·12	67·4	26	5	0·66	0·98	21	2·8	4·1
36	2·28	30·0	76	13	1·71	5·70	63	8·3	27·6
72	0·4	5·3	73	14	1·84	35·0	59	7·8	147·5

colour of the mature colonies. The total number of colonies in each bottle was estimated by dilution and plating of an aliquot from the control and treated series.

Since phenotypically indistinguishable *ad*-3 mutants originate by mutation at two separate but closely linked loci[2] (individual mutants can be identified as being *ad*-3*A* or *ad*-3*B* by heterokaryon tests), the mutation rates of two loci can be compared under the same conditions. The system also permits an investigation for specificity of mutagens since a comparison can be made of the proportion of the two types of *ad*-3 mutants after treatment of wild type with various types of mutagenic agents.

In an experiment for determining the rates of forward-mutation after X-ray treatment, aliquots of a conidial suspension were left untreated or irradiated with doses of 4·5, 18, 36 or 72 kr. Survival rates and the total number of purple mutants obtained with each treatment of the five aliquots of conidia are given in Table 1. No mutants were recovered from untreated conidia, and a total of 191 mutants were recovered from the four X-ray treatments. Genotypes of individual purple mutants were determined in subsequent heterokaryon experiments in which *ad*-3*A* and *ad*-3*B* tester strains were used. Since more purple mutants were recovered in this one experiment than in all other forward-mutation experiments combined[3], it was of interest to determine whether all such mutants were restricted to only the two known types: *ad*-3*A* or *ad*-3*B*. Since all the 191 mutants gave a positive heterokaryon test with one tester strain and a negative test with the other, all mutants were either *ad*-3*A* or *ad*-3*B*. No double *ad*-3*A* *ad*-3*B* mutants were recovered and no evidence was obtained for the presence of other loci giving phenotypically purple mutants in either the *ad*-3 region or elsewhere in the chromosome complement. Of the 191 purple mutants, 35 were *ad*-3*A* mutants and 156 *ad*-3*B* mutants.

A comparison of the total numbers of *ad*-3*A* mutants and *ad*-3*B* mutants gives a ratio of 1 : 4·5. This difference in the recovery of the two types of mutants was not unexpected since the combined data from numerous forward-mutation experiments in which the 'filtration-concentration' technique was used indicated an excess of *ad*-3*B* mutants. The present experiment provides direct evidence for a greater sensitivity to mutation as a result of X-irradiation of the *ad*-3*B* locus than the *ad*-3*A* locus. In Table 1, it can be seen that the induced rate of mutation of the *ad*-3*B* locus is about 4–5 times as high as the *ad*-3*A* locus at each dose level tested.

Although a microconidial strain was used in this experiment, the shape of the survival curve was not that expected[4] from a strain with only uninucleate conidia. The data suggest that a portion of the conidia was not uninucleate; thus the mutation rates for each locus were calculated on the basis of mutations per conidium, rather than in terms of mutations per nucleus or chromosome. By extension of the experimental points, maximum forward-mutation rates with this strain expected at a dose of 55 kr. are 9·7 and 2·1 per 10^6 viable conidia treated for the *ad*-3*B* and *ad*-3*A* locus, respectively.

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H. G. Kølmark

Biology Division,
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[1] Woodward, V. W., *Proc. U.S. Nat. Acad. Sci.*, **42**, 752 (1956).
[2] de Serres, F. J., *Genetics*, **41**, 668 (1956).
[3] de Serres, F. J., Case, M. E., Johnson, N. J., and Giles, N. H., unpublished.
[4] Giles, N. H., Cold Spring Harbor Symposia Quant. Biol., **16**, 282 (1951).

Some Observations on the Life-Cycle of *Atoxoplasma*, with Particular Reference to the Parasite's Schizogony and its Transmission by the Mite *Dermanyssus gallinae*

Since it was first shown that *Toxoplasma* infections may occur naturally in some birds (notably pigeons and chickens) there has been considerable speculation on the possible role of wild birds as host reservoirs for human *Toxoplasma* infections.

Although there have been many reports of *Toxoplasma* in a wide variety of wild birds, a large number of these observations are due to confusion of this parasite with hæmogregarines, schizonts of hæmogregarines, coccidial merozoites and exoerythrocytic stages of *Plasmodium*. One parasite even more frequently mistaken for *Toxoplasma* is *Atoxoplasma*[1]. This confusion is not surprising, when diagnosis is restricted to observations on the individual organism, for the two parasites are morphologically very similar. Their superficial resemblance to one another, failure to demonstrate any other stages in their life-cycles and the common habitat of both parasites in the cells of the lymphoid–macrophage system of the host have drawn many authors to the conclusion that *Atoxoplasma* and *Toxoplasma* are at least very closely related.

The following preliminary report is a summary of some recent studies of *Atoxoplasma* and clearly indicates that this parasite is much less closely related to *Toxoplasma* than was previously supposed.

The opportunity to study *Atoxoplasma* arose when I found 100 per cent of adult house sparrows (*Passer domesticus domesticus*) examined to be infected: these birds were trapped in the St. Albans area of Hertfordshire.

Later in the year it was established that young sparrows became infected, at an early age, while still in the nests: obviously, then, the mode of transmission was an extremely efficient one.

Large numbers of fledgling sparrows were trapped: all were infected, some very heavily. Many of these birds died in the aviary in which they were main-

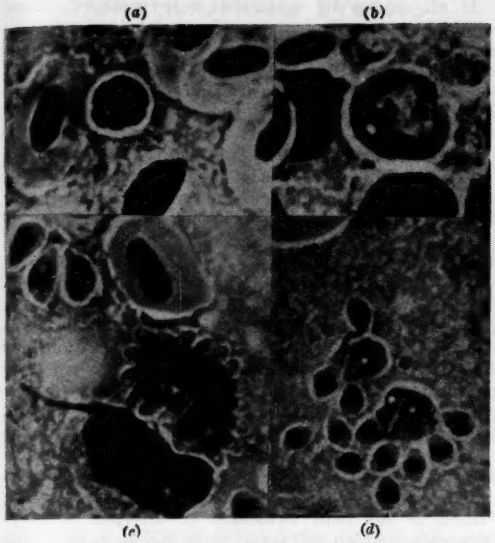

Fig. 1. (*a*) Early schizont of *Atoxoplasma* with three nuclei. (*b*) Schizont with ten nuclei. (*c*) Schizont, nearly mature, showing the peripheral distribution of the nuclei and finger-like protrusions of cytoplasm which form the merozoites. Compare size of merozoites with the normal *Atoxoplasma* seen in top left of same photomicrograph. (*d*) Mature schizont in process of splitting into merozoites; note residual cytoplasm

tained. Post-mortem examination of these birds revealed the spleens to be double their normal volume while the livers were enlarged, pale and frequently marbled in appearance. It was concluded that the tremendous numbers of atoxoplasms were responsible for the deaths.

A study of impression smears of spleen, liver and bone-marrow showed all stages of an undoubted schizogonic process of the parasite (Fig. 1, *a–d*). This occurs within the cells of the lymphoid–macrophage system, many of the schizonts becoming dislodged in the preparation of the smears. Up to 20–30 merozoites may be produced: these are substantially smaller than the normal atoxoplasm and possess a much more compact nucleus.

Atoxoplasma was passaged to canaries by the intraperitoneal inoculation of heavily infected, triturated sparrow tissues. Many of the infected canaries died, one with a very intense infection similar to that seen in the sparrows. The possibility of concomitant *Toxoplasma* infections being responsible for the sparrow and canary deaths was ruled out by the inoculation of mice, hamsters and guinea pigs, none of which became infected.

An apparent transmission of *Atoxoplasma* occurred when a canary was placed near another cage containing infected birds. As the only factor to account for this transmission was heavy infestation of the birds with 'red mite' (*Dermanyssus gallinae*) particular attention was focused on this ectoparasite.

Large numbers of mites from infected birds' cages were fed to a newly obtained canary. This bird was killed 14 days later and proved infected.

At present, attempts are being made to determine whether or not any development of *Atoxoplasma* takes place in the mites. So far it has only been possible to show that large numbers of the parasite can be found in crush smears of mites taken after feeding them on infected sparrows, but extended observations are necessary.

It seems likely that *Atoxoplasma* may be transmitted in Nature by mites. This suggestion is supported by the transmissions described above; also by the fact that *Dermanyssus gallinae* is commonly found in the nests of many wild birds (particularly sparrows) and young sparrows invariably become infected before they leave the nests.

The demonstration of a schizogonic phase of *Atoxoplasma* in the vertebrate host and the transmission of the parasite by mites now sharply differentiates *Atoxoplasma* from *Toxoplasma*.

A detailed account of these observations is to be published in the near future.

Added in proof. Since this communication was prepared, a number of naturally infected canaries have been found. Superficially this would appear to invalidate the experimental transmissions. As all canary breeders I have questioned admit to "inevitable presence of red-mite infestations in aviaries at some time or other", this ectoparasite must still remain highly suspect. The naturally infected canaries had been bred in outdoor aviaries, in the close vicinity of nesting sparrows.

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London, W.C.1. Sept. 26.

[1] Garnham, P. C. C., *Parasitology*, **40**, 328 (1950).

Perfect and Imperfect Density Dependence in Population Dynamics

In his recent article[1], Solomon has not made clear either my thesis or his reason for rejecting it. The thesis was developed in relation to my theory of natural control of insect populations for which a new classification of environmental factors was necessary[2,3]. Let me attempt to clarify.

An individual organism fulfils itself when it lives healthily, realizes its reproductive capacity fully, and dies of old age (senescence). Everything which helps or hinders fulfilment of the individual is the effective environment. Starting from Smith's[4] definition of a density-dependent factor, the effective environment may be classified as perfectly and imperfectly density-dependent factors and density-independent factors. There is only one perfectly density-dependent factor, intraspecific competition. The group named imperfectly density-dependent is comprised entirely of enemies (including competing species). Some enemies (for example, browsers, fruit eaters, etc.) come into the density-independent group of factors, a group which is otherwise well known as to its constituents. That is my position[2,3] very briefly.

In conversation and correspondence (October–December 1957) Solomon held, in substance, that since (1) intensity of intraspecific competition can be different for the same population number hence (2) this competition is not affected solely by numbers, that is, is not perfectly density-dependent. I argued that while (1) is correct, (2) is seen to be a *non-sequitur* as soon as it is realized that intraspecific competition is essentially a matter of environmental capacity. When intensity of competition is different for the same population number, this just means that environmental capacity is different. There is no getting away from the fact that for any given environmental capacity intraspecific competition is perfectly density-dependent. If the capacity alters, the curve of competition intensity changes in regard to the level of numbers for its origin and possibly changes

in regard to its slope, but it does not cease to be a function solely of numbers. It should be added that alteration of environmental capacity can be absolute, that is, independent of the organisms concerned, as when weather favours or retards the growth of vegetation for food or shelter; or it can be relative, that is, due to modification of average individual needs brought about, for example, by weather increasing and decreasing activity, or by some change in age-distribution or genetical composition of the population[2]. Mr. Solomon did not counter the above argument to my satisfaction.

Solomon now says[1]: "I do not believe that *factors* perfectly dependent on density exist except at a high level of abstraction. In Nature, the actions of all factors are presumably variable and inexact." [The italics are mine.]

In that statement it is interesting to note (1) a plurality not present in my thesis (cp. "factors"); and (2) an indirect admission that I am correct in holding enemies to be at best imperfectly density-dependent factors. I would suggest also that the "high level of abstraction" has actually existed for a long time in the unwitting ascription of perfect density-dependence to enemy action as in Nicholson's popular theory of natural control of population.

It is also interesting to note Solomon's adherence to another abstraction, namely, the 'inverse' density-dependent factor. This factor was a tentative proposition by Howard and Fiske[5], of which, as Allee *et al.*[6] point out, Smith[4] "made little". The proposition is on a par with the erroneous idea prevailing until recently[2,3] that a density-independent factor killed a constant proportion of the population in Nature. I put it to Mr. Solomon that there is no field evidence of 'inverse' density-dependence which will stand up to stringent ecological and statistical analysis (and that includes so-called 'sexual isolation').

Again there seems to me little point in distinguishing between factors the density-dependent action of which is (1) immediate ('direct') or (2) delayed ('alternate') simply because enemy reproduction is not immediate. I think it more useful to regard delay in action as contributing to the imperfection I ascribe to the density-dependence of enemies.

I should go further than Varley[7] (though for different reasons) and say there is no advantage in adopting Solomon's classification of density-dependent factors.

A. Milne

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Newcastle upon Tyne 1. July 23.

[1] Solomon, M. E., *Nature*, **181**, 1778 (1958).
[2] Milne, A., *Canad. Entom.*, **89**, 194 (1957).
[3] Milne, A., *Cold Spring Harbor Symposia on Quantitative Biology*, **22**, 253 (1957).
[4] Smith, H. S., *J. Econ. Entom.*, **28**, 873 (1935).
[5] Howard, L. O., and Fiske, W. F., *Bull. U.S. Bur. Ent.*, No. 91 (1911).
[6] Allee, W. C., Emerson, A. E., Park, O., Park, T., and Schmidt, K. P., "Principles of Animal Ecology", 331 (Saunders, Philadelphia and London, 1949).
[7] Varley, G. C., *Nature*, **181**, 1780 (1958).

I objected to Dr. Milne's proposed term "perfectly density-dependent" because neither intraspecific competition nor any other factor to be observed in the field is likely to preserve (in the words of his definition[1]) "an exact linear (or curvilinear) relationship between increasing action of the factor and increasing density of the species". The logic of his argument can be made clearer by re-stating it as follows.

If all causes of variation were removed, there would be a simple and exactly maintained relationship between the intensity of intraspecific competition and the population density. Let us relegate all sources of variation to a category for which we shall borrow the term environmental capacity. Then we are left with a perfectly density-dependent factor, intraspecific competition.

I see no objection to this argument as an exercise in logic or as a step in the development of a simplified mathematical theory. I only wish to point out that the perfection achieved in this way bears little relationship to what we can expect to find in studying the dynamics of actual populations, and that therefore the term seems inappropriate for use by the practical ecologist. It should perhaps be added that, by a similar process of abstraction, the relationships expected to hold between a parasite or predator population and the density of its host can also be made to appear simple and regular, as in the mathematical theories, although imperfect in Milne's sense.

Milne's remark about Nicholson's theory refers to a point already dealt with by Varley[2] in these columns.

Milne objects to the idea of inverse factors because "there is no field evidence of 'inverse' density-dependence which will stand up to stringent ecological and statistical analysis". But since, unfortunately, we have very little field information of this quality on density relationships of any sort, we can scarcely attach special significance to the lack of it in this case.

As to "the erroneous idea prevailing until recently that a density-independent factor killed a constant proportion of the population in Nature", in fact the idea that the action of density-independent factors varies in intensity is, of course, neither new nor at all unfamiliar. It is true that Howard and Fiske[5] wrote of catastrophic factors that "the average percentage of destruction remains the same, no matter how abundant or how near to extinction the insect may have become", and Smith[4], in re-naming these factors 'density-independent', described them as "destroying a constant percentage regardless of the abundance of the insect". But it seems obvious that their intention was to argue that the percentage effect of density-independent factors did not vary in response to changes in population density, not that they necessarily produced a mortality-rate which did not vary in time. In a discussion[5] published nine years ago, I unhesitatingly adopted the former view, and have seen no protest on this score. Milne's claim implies that ecologists have hitherto believed that mortality due to weather factors is constant.

Although Milne sees no advantage in my proposed classification of density-relationships, some ecologists may do so. It has at least the advantage of making no assumptions about particular types of factors in advance of our knowledge of them. This discipline is likely to be valuable for some time to come, even if the categories I suggested are augmented, subdivided or replaced by others.

M. E. Solomon

Pest Infestation Laboratory,
Department of Scientific and Industrial Research,
Slough, Bucks. Aug. 30.

[1] Milne, A., *Canad. Entom.*, **89**, 193 (1957).
[2] Varley, G. C., *Nature*, **181**, 1780 (1958).
[3] Howard, L. O., and Fiske, W. F., *Bull. U.S. Bur. Ent.*, No. 91 (1911).
[4] Smith, H. S., *J. Econ. Entom.*, **28**, 873 (1935).
[5] Solomon, M. E., *J. Anim. Ecol.*, **18**, 1 (1949).

[This correspondence is now closed.—Editors, *Nature*.]

FORTHCOMING EVENTS

*(Meetings marked with an asterisk * are open to the public)*

Monday, November 3

UNIVERSITY OF LONDON (in the Physiology Theatre, Gower Street, London, W.C.1), at 5 p.m.—Dr. J. R. Postgate: "The Sulphur Bacteria".* (Further lecture on November 10.)

INSTITUTE OF PETROLEUM (at 61 New Cavendish Street, London, W.1), at 5.30 p.m.—Mr. A. F. Fox: "Some Problems of Petroleum Geology in Kuwait".

ROYAL GEOGRAPHICAL SOCIETY (at 1 Kensington Gore, London, S.W.7), at 5.30 p.m.—"African Safari" (colour film with commentary by Lewis Cotlow).

SOCIETY OF ENGINEERS (at the Geological Society, Burlington House, Piccadilly, London, W.1), at 5.30 p.m.—Capt. G. S. Ritchie, D.S.C., R.N.: "Hydrography".

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (at Norwood Technical College, Knight's Hill, West Norwood, London, S.E.27), at 7 p.m.—Dr. J. F. Padday: "The Use of Photography in Scientific Investigations".

Tuesday, November 4

UNIVERSITY OF LONDON (at Birkbeck College, Malet Street, London, W.C.1), at 4 p.m.—Dr. E. W. Gorter (Eindhoven): "The Orientation of the Magnetic Moments in Ferrimagnetic Oxides with Respect to Each Other and to the Crystal Axes".*

UNIVERSITY OF LONDON (at the London School of Economics and Political Science, Houghton Street, London, W.C.2), at 5 p.m.—Prof. W. F. Wertheim (Amsterdam): "Sociological Aspects of Inter-island Migration in Indonesia".*

UNIVERSITY OF LONDON (at Bedford College, Regent's Park, London, N.W.1), at 5.15 p.m.—Prof. H. G. Eggleston: "The Concept of Convexity"* (Inaugural Lecture).

INSTITUTION OF ELECTRICAL ENGINEERS, MEASUREMENT AND CONTROL SECTION (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. R. C. M. Barnes and Dr. J. H. Stephen: "Operating Experience with a Transistor Digital Computer"; Mr. D. Eldridge: "A New High-Speed Digital Technique for Computer Use".

UNIVERSITY OF LONDON (in the Gustave Tuck Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Mr. J. F. Coales: "Automatic Control".* (Further lectures on November 6, 11 and 13.)

UNIVERSITY OF LONDON (in the Physiology Theatre, University College, Gower Street, London, W.C.1), at 5.30 p.m.—Prof. J. F. Danielli, F.R.S.: "A Generalized Cell Theory and Its Relevance to Certain Problems of Drug Action".* (Further lectures on November 11 and 18.)

ROYAL AERONAUTICAL SOCIETY (at 4 Hamilton Place, London, W.1), at 7 p.m.—Mr. W. N. Neat: "The Spectre Rocket Engine".

Wednesday, November 5

UNIVERSITY OF LONDON (at the Postgraduate Medical School of London, Ducane Road, London, W.12), at 2 p.m.—Dr. G. M. McGowan: "Potassium Metabolism".*

PHYSICAL SOCIETY, COLOUR GROUP (in the Lecture Theatre, Institute of Ophthalmology, Judd Street, London, W.C.1), at 3.30 p.m.—Mr. F. S. Said: "The Transmissivity of the Living Human Crystalline Lens". 5 p.m.—Films: "This is Color" and "Color Magic".

SOCIETY FOR ANALYTICAL CHEMISTRY (at the Chemical Society, Burlington House, Piccadilly, London, W.1), at 7 p.m.—Scientific Papers.

ROYAL METEOROLOGICAL SOCIETY (at 49 Cromwell Road, London, S.W.7), at 7.45 p.m.—Dr. B. J. Mason: "Recent Developments in the Physics of Rain and Rainmaking".

Thursday, November 6

UNIVERSITY OF LONDON (in the Anatomy Theatre, University College, Gower Street, London, W.C.1), at 1.15 p.m.—Dr. D. Wilkie: "The Aims of Sexual Reproduction".*

ROYAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 4.30 p.m.—Dr. J. Needham, F.R.S.: "The Missing Link in Horological History—a Chinese Contribution" (The Wilkins Lecture).

LINNEAN SOCIETY OF LONDON (at Burlington House, Piccadilly, London, W.1), at 5 p.m.—Dr. John Ramsbottom, O.B.E.: "Linnaeus, *Pan Suecica* and Nomenclature"; Dr. H. Hamshaw Thomas, M.B.E., F.R.S.: "Angiosperm Evolution—Historical and Ecological Considerations".

INSTITUTE OF REFRIGERATION (at the Institute of Marine Engineers, The Memorial Building, 76 Mark Lane, London, E.C.3), at 5.30 p.m.—Sir Rupert de la Bère, Bart., K.C.V.O.: Presidential Address.

INSTITUTION OF ELECTRICAL ENGINEERS (at Savoy Place, London, W.C.2), at 5.30 p.m.—Mr. T. S. Pick and Mr. A. Readman: "The Recognition of Moving Vehicles by Electronic Means".

ROYAL ANTHROPOLOGICAL INSTITUTE OF GREAT BRITAIN AND IRELAND (at 21 Bedford Square, London, W.C.1), at 5.30 p.m.—Miss Kathleen M. Kenyon, C.B.E.: "Some Observations on the Beginnings of Settlement in the Near East".

UNIVERSITY OF LONDON (at Queen Elizabeth College, Campden Hill Road, London, W.8), at 5.30 p.m.—Sir Vivian Fuchs: "The Trans-Antarctic Expedition".

INSTITUTION OF MECHANICAL ENGINEERS, INTERNAL COMBUSTION ENGINE GROUP (at 1 Birdcage Walk, Westminster, London, S.W.1), at 6 p.m.—Discussion on "Do We Know Enough About the Induction and Exhaust Processes?"

SOCIETY OF CHEMICAL INDUSTRY, MICROBIOLOGY GROUP (at 14 Belgrave Square, London, S.W.1), at 6.15 p.m.—Dr. Herchel Smith: "Investigations of the Biosynthesis of some Mould Metabolites using Radioactive Tracers".

INSTITUTE OF METALS (at the Royal School of Mines, South Kensington, London, S.W.7), at 7 p.m.—Prof. J. G. Ball: "Experimental Features of Investigations with Radioactive Materials".

POLAROGRAPHIC SOCIETY (at "The Duke of York", 8 Dering Street, London, W.1), at 7 p.m.—Informal Discussion Meeting on "Some Problems Encountered in the Polarography of Metallic Cations in the Presence of Bis-Quaternary Compounds" introduced by Dr. P. O. Kane.

ROYAL INSTITUTE OF CHEMISTRY, LONDON SECTION (joint meeting with the Brunel College of Technology Chemical Society, at the Brunel College of Technology, Woodlands Avenue, Acton, London, W.3), at 7 p.m.—Prof. D. P. Craig: "Theory and Experiment in Chemistry".

CHEMICAL SOCIETY (at Burlington House, Piccadilly, London, W.1), at 7.30 p.m.—Sir Eric Rideal, F.R.S.: Irving Langmuir Memorial Lecture.

Thursday, November 6—Friday, November 7

INSTITUTE OF WELDING (at 54 Princes Gate, Exhibition Road, South Kensington, London, S.W.7), at 10 a.m. daily—Autumn Meeting.

Friday, November 7

MINERALOGICAL SOCIETY, CLAY MINERALS GROUP (at the Geological Society of London, Burlington House, Piccadilly, London, W.1), at 10.45 a.m.—Scientific Papers. 5 p.m.—Annual General Meeting.

ASSOCIATION OF APPLIED BIOLOGISTS (in the Lecture Hall of the British Museum (Natural History), Cromwell Road, London, S.W.7), at 10.50 a.m.—Symposium on "Clubroot Disease".

COUNCIL FOR THE PRESERVATION OF RURAL WALES (at the Church House, Welshpool, Montgomeryshire), at 2.30 p.m.—Annual General Meeting. Speakers: The Lady Megan Lloyd George, LL.D., J.P., M.P., the Rt. Hon. Clement Davies, P.C., Q.C., M.P.

ROYAL SOCIETY OF MEDICINE, ANÆSTHETICS SECTION (at 1 Wimpole Street, London, W.1), at 5.30 p.m.—Prof. J. H. Burn: "The Pharmacological Investigation of Anæsthetic Drugs for Clinical Trial".

INSTITUTION OF ELECTRICAL ENGINEERS, MEDICAL ELECTRONICS DISCUSSION GROUP (at Savoy Place, London, W.C.2), at 6 p.m.—Discussion Meeting on "Problems in the Measurement of Blood Flow". Opening Speakers: Dr. A. C. Dornhorst and Dr. D. A. McDonald.

SOCIETY OF DYERS AND COLOURISTS, LONDON SECTION (at the Royal Society, Burlington House, Piccadilly, London, W.1), at 6 p.m.—Mr. S. M. Jaeckel and Mr. D. L. Munden: "Some Aspects of the Dyeing and Finishing of Fabrics Knitted from Bulky and Stretched Yarns".

SOCIETY OF CHEMICAL INDUSTRY, FINE CHEMICALS GROUP (at 14 Belgrave Square, London, S.W.1), at 6.30 p.m.—Dr. A. Cohen: "Synthesis in the Ergot Alkaloid Field".

ROYAL AERONAUTICAL SOCIETY (joint meeting with the HELICOPTER ASSOCIATION, at 4 Hamilton Place, London, W.1), at 7 p.m.—Dr. G. S. Hislop: "Development of the Fairey Rotodyne".

Saturday, November 8

LONDON COUNTY COUNCIL (at the Horniman Museum, London Road, Forest Hill, London, S.E.23), at 3.30 p.m.—Prof. C. von Fürer Haimendorf: "Buddhist Society in the Himalayas".*

APPOINTMENTS VACANT

APPLICATIONS are invited for the following appointments on or before the dates mentioned:

ASSISTANT (with special qualifications in organic chemistry) IN THE DEPARTMENT OF CHEMISTRY—The Secretary, The University, Edinburgh (November 8).

HEAD (with a good honours degree and experience of teaching in an institution of higher education) OF THE DEPARTMENT OF SCIENCE, Nigerian College of Arts, Science and Technology—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (November 10).

LECTURER IN RADIO-ASTRONOMY—The Registrar, The University, Manchester 13 (November 10).

SENIOR LECTURER (with a good honours degree and teaching experience) IN APPLIED MATHEMATICS; and LECTURERS (2) (with a good honours degree and teaching experience) IN MATHEMATICS at the Nigerian College of Arts, Science and Technology—The Council for Overseas Colleges, 12 Lincoln's Inn Fields, London, W.C.2 (November 10).

ASSISTANT LECTURER IN THE DEPARTMENT OF PURE MATHEMATICS—The Registrar, University College of Wales, Aberystwyth (November 15).

ASSISTANT LECTURER (with an honours degree from a recognized university and preferably previous teaching or research experience) IN PSYCHOLOGY at the University of Canterbury, Christchurch, New Zealand—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (New Zealand, November 15).

LEVERHULME STUDENT (with a postgraduate diploma in chemical engineering or equivalent qualifications) IN CHEMICAL ENGINEERING—The Registrar, King's College (University of London), Strand, London, W.C.2 (November 17).

SENIOR LECTURER or LECTURER IN PHYSICS—The Registrar, The University, Manchester 13 (November 17).

LECTURER IN THE DEPARTMENT OF MATHEMATICAL PHYSICS—The Secretary, The University, Edinburgh (November 24).

HEAD (with a degree in applied mathematics) OF THE DEPARTMENT OF MATHEMATICS AND PHYSICS, Kumasi College of Technology—The Council for Overseas Colleges of Arts, Science and Technology, 12 Lincoln's Inn Fields, London, W.C.2 (November 25).

CHAIR OF MECHANICAL ENGINEERING—The Secretary, The Queen's University, Belfast (November 30).

RESEARCH FELLOW IN HEAT TRANSFER, to pursue research into the mechanism of heat transfer from long pipes, carrying steam in the transition region from superheated to saturated—The Registrar, University College of Swansea, Singleton Park, Swansea (December 1).

SENIOR LECTURER IN MECHANICAL ENGINEERING—The Secretary, The Queen's University, Belfast (December 1).

LECTURER IN EDUCATION at the University College of Rhodesia and Nyasaland—The Secretary, Inter-University Council for Higher Education Overseas, 29 Woburn Square, London, W.C.1 (December 5)

PROFESSOR IN PHYSIOLOGY at the University of Queensland, Australia.—The Secretary, Association of Universities of the British Commonwealth, 36 Gordon Square, London, W.C.1 (Australia, December 15).

LECTURER and an ASSISTANT LECTURER IN GENETICS—The Secretary of University Court, The University, Glasgow (December 31).

DIRECTOR OF THE COMMONWEALTH BUREAU OF HORTICULTURE AND PLANTATION CROPS, East Malling Research Station, near Maidstone, Kent—The Secretary, Commonwealth Agricultural Bureaux, Farnham Royal, Bucks (January 31).

ASSISTANT LECTURER, Grade B (with a degree or equivalent and preferably industrial and/or teaching experience), IN TELECOMMUNICATIONS, to teach up to the Final Certificate of the City and Guilds of London Institute and the R.T.E.B. Radio and Television Servicing Certificates—The Clerk to the Governors, Northern Polytechnic, Holloway, London, N.7.

ASSISTANT (preferably with experience of biochemical or other techniques in microbiology), for research on the mode of action of antituberculous drugs—The Secretary, Guy's Hospital Medical School, London, S.E.1.

FELLOW IN THE DEPARTMENT OF CHEMISTRY, for fundamental research on electron resonance spectroscopy—The Registrar, The University, Birmingham 15.

JUNIOR BACTERIOLOGIST, to assist in the investigation of intestinal disorders of newborn animals—The Secretary, Royal Veterinary College (University of London), Royal College Street, London, N.W.1.

LABORATORY ASSISTANT (age about 30–35, experience of anatomical and biological laboratory techniques, and preferably an elementary knowledge of photographic processes and/or histological procedures)—The Establishments Officer, Zoological Society of London, Regent's Park, London, N.W.1.

LECTURERS (2) (graduates of a British university and preferably specialists in experimental microwave physics or theoretical metal physics) IN PHYSICS—The Clerk to the Governing Body, Battersea College of Technology, London, S.W.11.

PROFESSOR (British subject with British university qualifications) OF BIOCHEMISTRY in the University of Teheran, Iran—The Director, Personnel Department, British Council, 65 Davies Street, London, W.1, quoting N.3(58).

RESEARCH FELLOW (with a good research background and suitably qualified academically) IN METALLURGY OR SOLID STATE PHYSICS in the Department of Aircraft Materials—The Recorder, College of Aeronautics, Bletchley, Bucks.

RESEARCH PHYSICISTS (with a first- or good second-class honours degree) for (*a*) to exploit techniques for measuring flux and spectral distribution of neutrons from reactors and accelerators; and (*b*) to study and measure high energy (greater than 10 MeV.) radiations—The Group Recruitment Officer (1233/34), U.K.A.E.A., Atomic Energy Research Establishment, Harwell, Berks.

TECHNICAL ASSISTANT IN ELECTRONICS in the Isotopes Laboratory, for work which will involve the maintenance of routine counting equipment, as well as the designing and building of apparatus to research requirements—Joint Clerk to the University Court, Queen's College, Dundee.

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